Synergy™

Personal Peptide Synthesizer

User Manual

Applied Biosystems

For Research Use Only. Not for use in diagnostic procedures.

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Synergy User's Manual Conventions

User Attention Words

Throughout the Synergy Installation Guide and the Synergy User's Manual, four kinds of information are set off from the regular text. Each "user attention word" requires a particular level of observation or action that is significant to user safety or proper instrument operation.

Note	***Used to call attention to information.***

IMPORTANT	***Indicates information that is necessary for proper instrument operation.***

Caution	***Damage to the instrument could result if you do not comply with this information.***

WARNING	***Physical injury to the user or other persons could result if these precautions are not implemented.***

Chemical abbreviations

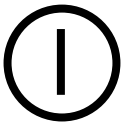
The following chemical abbreviations are used throughout the Synergy Installation Guide and the Synergy User's Manual.

DIEA	N,N-diisopropylethylamine
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
EDT	1, 2, ethanedithiol
HBTU	2-(1 H-benzotriazol-1-yl)-1, 1, 3, 3-tetra-methyluronium hexafluorophosphate
HOBt	1-Hydroxybenzotriazole
MTBE	methyl *t*-butyl ether
NH_4OH	ammonium hydroxide
NMP	N-methylpyrrolidone
TFA	trifluoroacetic acid
THF	tetrahydrofuran
PIP	piperidine

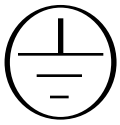
Synergy Safety Symbols

The symbols shown here are affixed to the Synergy Peptide Synthesizer. Read the explanations and make sure you understand what the symbols mean before you interact with the instrument in any way.

Electrical Symbols



This symbol indicates the on and off positions of a push-push main power switch.



This protective grounding terminal must be connected to earth ground before any other electrical connections are made to the instrument.

This terminal either receives or delivers alternating current or voltage.

This terminal can receive or supply an alternating and a direct current or voltage.

This symbol indicates the presence of high voltage.

Other

This symbol appears on the instrument to indicate that further information is available in this Safety supplement. The information corresponding to these labels on Synergy is in the following section.

Synergy Safety Procedures

General Laboratory Safety

Your laboratory should have all equipment required for the safety of individuals working with chemicals including fire extinguishers, first-aid equipment, safety shower and eye-wash fountain, and spill cleanup equipment.

Instrument Access

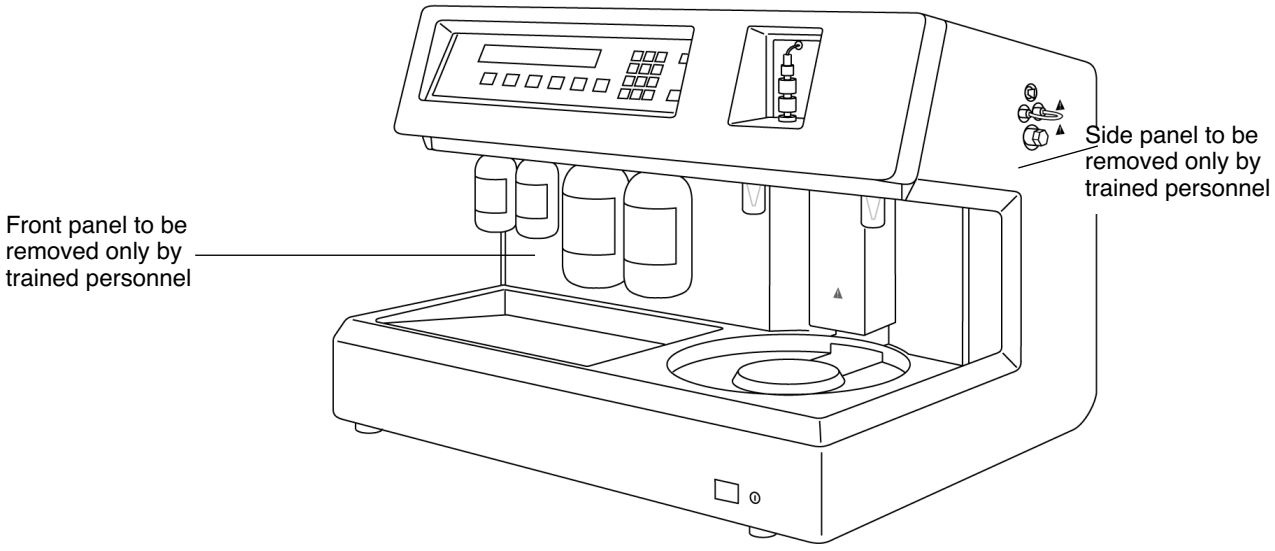


Figure 1-1. *Front view of Synergy*

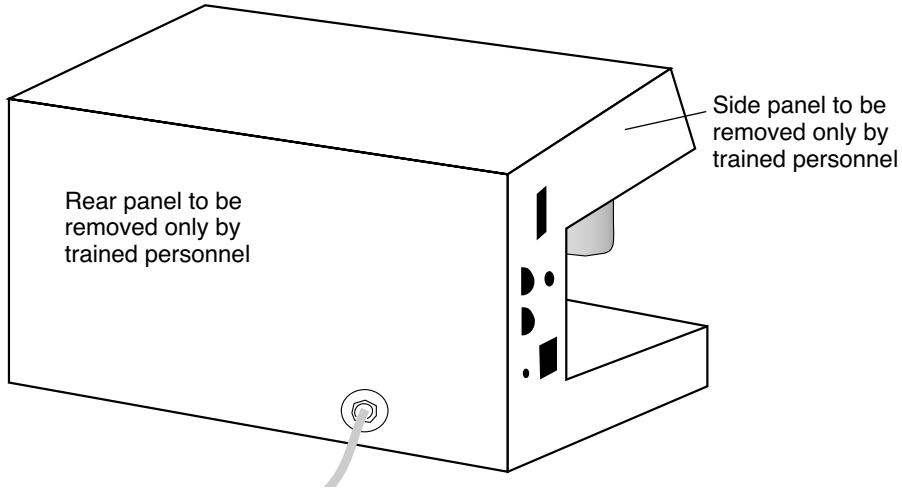


Figure 1-2. *Rear view of Synergy*

Figure 1-1 and Figure 1-2 illustrate instrument accessibility.

Fuses

For continued protection against the risk of fire, replace fuses only with listed and certified fuses of the type and rating specified on the rear of the instrument.

Jaw Assembly

The jaw assembly is operated by a pneumatic valve under high pressure. Keep fingers away from the jaws during the turntable self-test and whenever the turntable is in motion.

Hazardous Chemicals

This instrument contains hazardous chemicals in bottles, lines and valve blocks. Do not operate or interact with the instrument until you have read all the Material Safety Data Sheets (MSDSs) for chemicals associated with this instrument.

Table 1-1 lists the chemicals used on Synergy.

Table 1-1. Chemicals for *FastMoc* Chemistry on Synergy

Bottle	Volume (mL)	Chemical
A	40	0.2 M 2-(1H-benzotriazol-1-yl)-1, 1, 3, 3-tetramethyluronium hexafluorophosphate)/1-hydroxybenzotriazole/Dimethyl sulfoxide/N-methylpyrrolidone
B	40	0.4 M N, N-Diisopropylethylamine/Dimethyl sulfoxide/ N-methylpyrrolidone
1	200	Piperidine
2	200	Tetrahydrofuran
10	4000	Dimethylformamide

The MSDSs for hazardous chemicals supplied by Applied Biosystems are presented after the Safety Data tab of this User's Manual.

Waste Bottle

This instrument produces hazardous, liquid and gaseous waste. Ensure that the waste container is correctly installed, as shown on page 23 of the Installation Guide. Do not dispose of hazardous waste until you have read the Synergy Waste profile found after the Safety Data tab of this User's Manual.

WARNING	**HAZARDOUS WASTE** ***The Waste Profile gives information on composition, physical properties, reactivity, spill and leak cleanup, health, fire and explosion hazards, waste disposal, special protective equipment and precautions. Dispose of hazardous waste in accord with all local, state, and federal regulations. The Synergy Waste Profile can be found after the Safety Data tab of this User's Manual.***

Secondary Containment

WARNING	**HAZARDOUS WASTE** ***Synergy generates hazardous chemical waste. Place the external waste bottle in the safety carrier provided for all hazardous materials and waste. Read the Synergy Waste Profile, found after the Safety Data tab of this User's Manual.***

Compressed Gas Cylinders

If the compressed gas cylinder is not properly secured, it could fall over and explode, which could cause physical injuries.

WARNING	**GAS TANK EXPLOSION HAZARD** ***Pressurized gas cylinders are explosive. Attach pressurized gas cylinders to a wall or bench by means of approved brackets or chains. Always cap the gas cylinder when not in use.***

External Pneumatic Supply

Synergy requires 60-75 psi nitrogen input for operation.

Laboratory Ventilation Requirements

The information presented here reflects U.S. regulations and practices for venting all Applied Biosystems, Inc. instruments to a fume hood or to a duct.

WARNING **POTENTIAL FOR EXPOSURE TO HAZARDOUS WASTES** ***Do not operate a vented instrument unless it is connected in accordance with all the ventilation requirements presented here. The wastes produced by certain chemicals in Applied Biosystems instruments are hazardous and can cause injury, illness, or death. Always wear protective clothing when handling hazardous waste. Mix and prepare hazardous materials beneath a fume hood. Read the Waste Profile in Appendix 2.***

Fume Hood

Always mix and prepare hazardous materials beneath a fume hood. The vent line to the fume hood should be no longer than 15 feet (5 meters). Be certain that the line is not punctured or otherwise damaged.

Following are important points about the fume hood:

- It should operate at all times, including nights and weekends, when vented waste bottle contents can escape to surroundings.
- It is to be located away from air currents generated by air conditioning ducts, fans, windows, doors, and moving equipment and persons.
- It should exhibit a sign or label indicating where doors and windows must be positioned to give an average flow of 100 ft/min (linear) face velocity. The minimum velocity of flow at any point in the hood must be 80 ft/min (linear); the maximum flow velocity must not exceed 125 ft/min.
- It must meet local, state, and federal health and safety requirements. In addition, see current fume hood standards established by the American Society of Heating, Refrigeration, Air Conditioning Engineers (ASHRAE); American Conference of Governmental Industrial Hygienists (ACGIH); and Occupational Safety and Health Agency (OSHA).
- It must be constructed of materials that are compatible with the waste materials/chemicals being generated/exhausted.

Figure 1-3 shows a fume hood configuration with movement of gaseous waste through the hood into a duct (the duct is described in the following section).

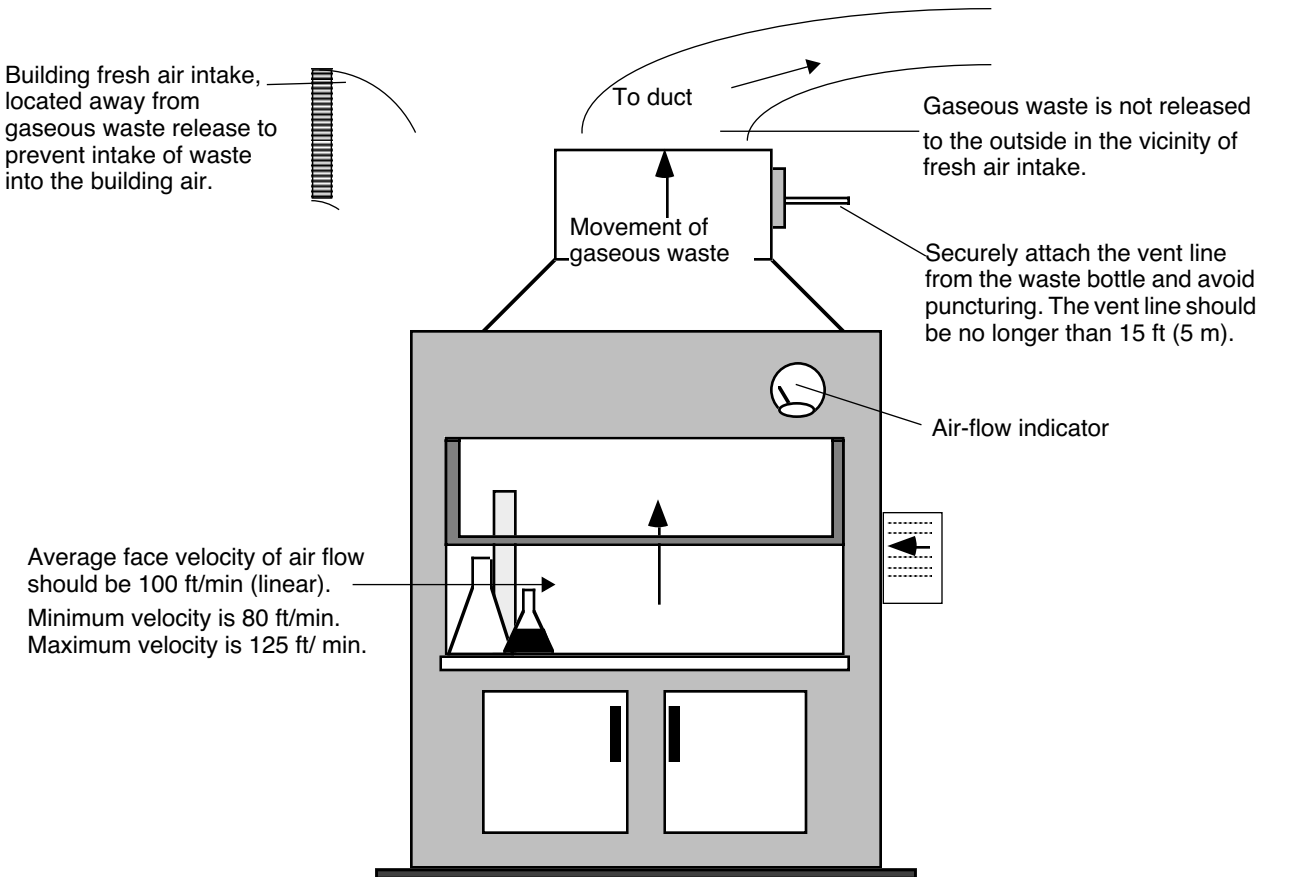


Figure 1-3. *Venting Gaseous Waste Produced by Instrument to a Fume Hood*

Routine Maintenance Tasks

- Periodically (at least twice a year) check and record face velocity. If results fail to meet standards, make ventilation adjustments as soon as possible to prevent employee exposure to hazardous fumes.
- Periodically (at least once a year) inspect and maintain exhaust system, including fans and motors.

Duct System

Gaseous waste is vented through a fume hood to a duct, or is directly vented through the duct. The following are important points about U.S. regulations and practices related to the duct:

Duct Work

- Should be constructed of materials compatible with the waste materials being generated.

- Should operate at all times, including nights and weekends, when vented waste bottle contents can escape to surroundings.
- Should not come in contact with strong oxidizers, bases, or other chemicals that are incompatible with gaseous waste.
- Should allow vapor or gas movement 1000–2000 fpm.

Tubing

- Do not let the open end of tubing face into oncoming air movement through duct or canopy.
- Keep away from sources of potential damage such as contact, heat, or flame.
- Place the tubing end as far as possible into the duct, canopy, or hood.
- Use polypropylene tubing of the shortest possible length and straightest possible run. Tubing length should not exceed 15 feet (5 meters).
- Eliminate low points that can trap residue or condensation.
- Fasten tubing securely. Use fasteners of polypropylene or teflon. Do not use brass; it corrodes. Be careful not to puncture tubing.

Canopy

- Operate air exhaust at all times, including nights and weekends, when vented waste bottle contents can escape to surroundings.

WARNING **POTENTIAL EXPOSURE TO HAZARDOUS GASEOUS WASTE**
Do not connect the waste vent to a ductless hood or to a system that purifies, filters air and returns it to the room. See the Synergy Waste Profile after the Safety Data tab in this User's Manual.

Figure 1-4 shows a duct system and fume hood canopy with tubing connected to the instrument waste exhaust line.

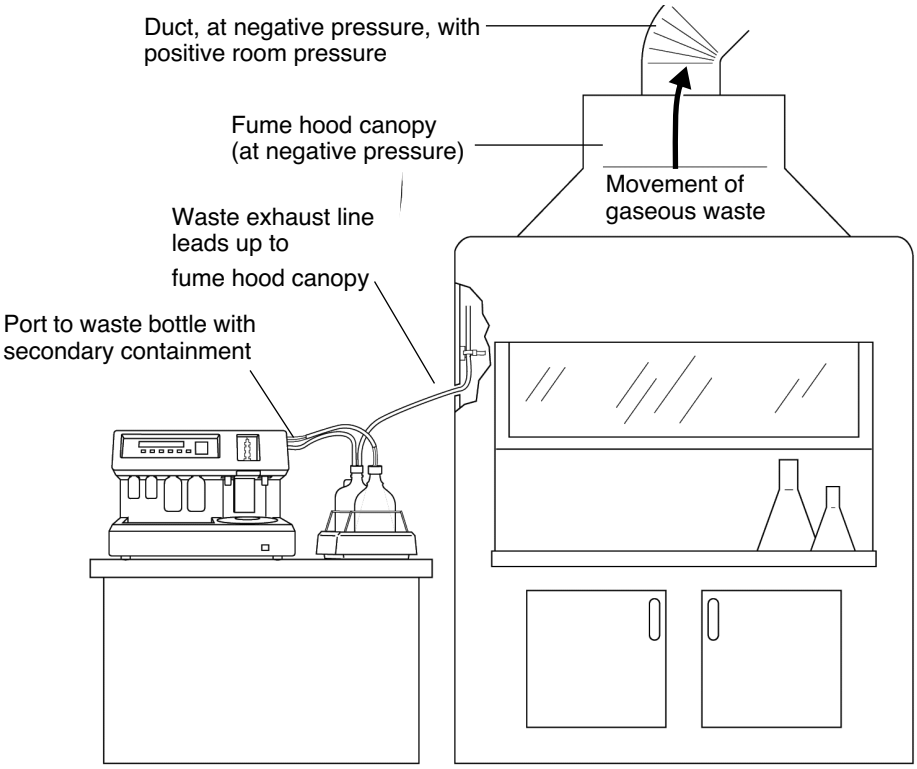


Figure 1-4. *Venting gaseous waste directly to duct*

Routine Maintenance Tasks

- Periodically (at least once a year) inspect and maintain fans and motors.
- Visually inspect tubing periodically for breakage, crimping, corrosion (if metal), or other damage. Replace the tubing as necessary.
- Flush the vent line with pure, dry pressurized gas to remove buildup of condensation or particulates. Handle and dispose of all wastes as if they were hazardous.

WARNING	**POTENTIAL EXPOSURE TO HAZARDOUS WASTE** ***Always wear a laboratory coat, eye protection, and gloves that resist waste chemicals when you work on waste bottles, exhaust lines/tubing, and liquid traps.***

2 Routine Operation

This section of the manual describes how you can use Synergy to perform routine peptide syntheses. Before you follow the procedures in this section, your instrument should be installed and should have already synthesized the test peptide, LAGV, as described in the Synergy Installation Guide.

Contents

A Brief Description of Synergy

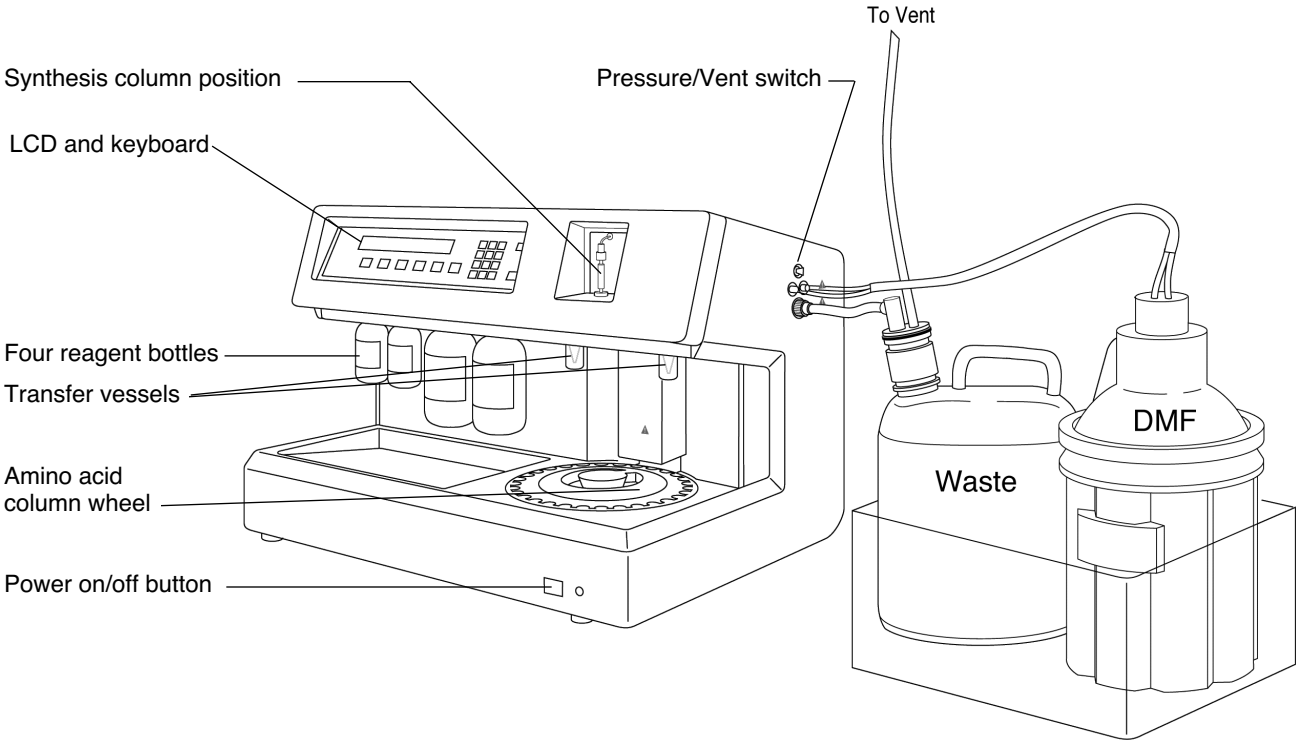


Figure 2-1. *Synergy, after installation*

The Applied Biosystems Synergy Peptide Synthesizer combines Fmoc (9-fluorenylmethylcarbonyl) synthesis chemistry with HBTU [2-(1-H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] activation to perform solid-phase peptide synthesis (SPPS). A unique system of gas-pressurized valves, reagent reservoirs, and transfer vessels directs reagent flow through three stages of synthesis:

- Deprotection of N-terminal amino acid of peptide bound to resin support in the peptide synthesis column.
- Activation of amino acid in column on wheel.
- Transfer of activated amino acid to the peptide synthesis column, where coupling with N-terminal amino acid occurs.

The Synergy software monitors the conductivity of the solution passing through the peptide synthesis column (PSC) and determines the reaction times of both deprotection and coupling. With the printing option, users may observe a real-time, graphic representation of each deprotection-coupling cycle as synthesis progresses.

The LCD and Keyboard

With the liquid crystal display (LCD) and keyboard, you can monitor each step of instrument operation and select software menus to perform instrument system tests or modify pre-programmed lines.

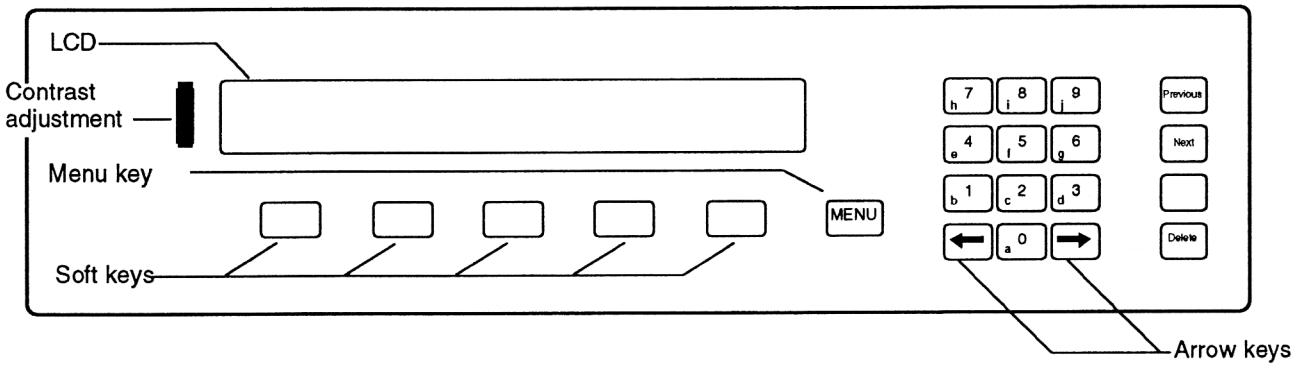


Figure 2-2. *The Synergy LCD and keyboard*

The LCD on the front of Synergy displays messages on two lines of 40 characters each. Usually, the top line displays information or directions. The bottom line assigns names to the soft keys located directly under the LCD.

A **cursor**, represented by a blinking, horizontal line (—) on the LCD, appears under text that you can modify.

Five soft keys under the LCD correspond to choices displayed on the bottom line of the LCD. The ***HOLD*** and ***PAUSE*** soft keys are like toggle switches. When you press either the ***HOLD*** or ***PAUSE*** soft key once, an asterisk (*****) appears next to the key to indicate the key is operating or "on." Press the key again, and the asterisk goes away to indicate the key is "off."

Sixteen labeled keys to the right of the LCD let you interact with Synergy software controller by way of the LCD.

- Use the **alphanumeric keys** to enter numbers on the LCD. In some menus, these keys can represent lower case letters (**a, b, c,** etc.) or upper case letters (**A, B, C,** etc.).
- Use the **arrow** keys (←→) to move the cursor on the LCD to the left or to the right.
- Use the **Previous** and **Next** keys to increase or decrease the value currently selected by the cursor.
- Use the **Delete** key to erase the entry that is currently selected by the cursor.
- Press the **Menu** key to see the preceding LCD display and to return to the Main Menu.

Making a Peptide on Synergy

With Synergy, every step of solid-phase peptide synthesis has been incorporated into a series of pre-programmed module lines (Table 2-1). The conductivity monitoring feature automatically optimizes deprotection and coupling to allow synthesis of many peptides without modifying these module lines. See Chapter 5 for a detailed explanation of automated peptide synthesis on Synergy.

Table 2-1. Pre-programmed Synthesis Lines on Synergy

Line Name	Modules	Action
BEG	b f	initial resin solvation
L (cycle line) - 1	j d a c g f i	deprotection and coupling
END	d e	Fmoc deprotection and dry peptide-resin

This chapter describes the three stages of a peptide synthesis with these pre-programmed module lines on Synergy.

1. Preparing Synergy for synthesis (page 2-5)
2. Selecting and loading the columns for your synthesis (page 2-10)
3. Starting automated synthesis (page 2-14)

Prepare Synergy for Synthesis

Synergy requires four visual checks to confirm the instrument is ready for operation.

1. Check the external gas supply.
2. Check levels of 5 reagent bottles.
3. Check the waste bottle and empty it, if necessary.
4. Check the printer power, connections, and paper supply.

1. Check the external gas supply

Regulated pressure: 60 - 70 psi **Gas tank pressure:** ≥ 300 psi

Pressurized gas controls reagent flow and the action of the autosampler jaws that close on the AACs during synthesis. Without an adequate gas supply, Synergy ceases operation (see ***Empty Gas Tank*** on page 7-10). To maintain adequate gas pressure, we recommend the nitrogen tank pressure (high pressure gauge) should not drop below 300 psi and the low pressure gauge should be set at 60 - 75 psi.

WARNING	**GAS TANK EXPLOSION HAZARD.** ***Pressurized gas cylinders are explosive. Attach pressurized gas cylinders firmly to a wall or bench by means of approved brackets, chains, or clamps. Always cap the gas cylinder when not in use.***

How to change the external gas tank

1. Switch the Pressure/Vent switch on Synergy to Vent (Figure 2-1) and wait one minute for the system to depressurize.
2. Close both the main supply valve on top of the gas tank and the needle valve, if one exists, on the tank regulator.
3. Remove the regulator from the empty tank and install it on a full replacement tank. Use only pre-purified compressed nitrogen gas on Synergy. You do not need to disconnect either end of the tubing that connects the regulator to Synergy.
4. Open the main tank supply valve. Squirt soapy water around the connection between the regulator and the gas tank to check for leaks.
5. Verify that the tank regulator setting returns to its original position.
6. If the regulator has a needle valve, open it completely.
7. Switch the Pressure/Vent switch to Pressure.

2. Check levels of 5 reagent bottles

WARNING	**POTENTIAL CHEMICAL HAZARD.** ***Regard all chemicals on the synthesizer, including liquid in the lines, as potentially hazardous. Wear protective eyeglasses, gloves and a laboratory coat when working with the chemicals used on the instrument. If a spill occurs, clean it up in accordance with instructions in the MSDSs or Waste Profile found in the Pre-Installation Manual and in the Safety Supplement of the Synergy User's Manual.***

Table 2-2 lists the 5 reagent bottles found on Synergy with average expected usage. You can expect the values on this table associated with HBTU and DIEA to remain constant, regardless of which amino acids go into your peptide synthesis. However, piperidine and DMF usage vary as the deprotection reactions vary in length.

Table 2-2. Synergy Approximate Reagent Usage

Reagent	Bottle size (mL)	mL/cycle (approx.)	mm/cycle (approx.)	Cycles/bottles
HBTU	40	0.4	0.5	100
DIEA	40	0.4	0.5	100
Piperidine	200	2	1.0	100
THF	200	2	1.0	100
DMF	4000	60	3*	67

* DMF mm/cycle value applies to that part of the reagent bottle below the neck, where the bottle sides do not taper.

You may find it helpful to place a vertical strip of white tape on the side of each reagent bottle to track reagent usage. After each synthesis, place a horizontal mark on the tape that corresponds with the current level of reagent in each bottle. The values in the column labeled "mm/cycle" in Table 2-2 reflect the average change in the reagent level, measured in millimeters, per cycle.

Use Table 2-2 and the number of amino acids in your next peptide to estimate how much reagent you will need to complete the synthesis. If necessary, **replace reagent bottles before synthesis begins** (see ***Bottle Changing Procedure*** on page 4-9).

You must combine the contents of two reagent bottles to prepare the HBTU 2-(1-H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] reagent. Instructions for preparing the HBTU reagent follow. The prepared reagent is good for six weeks.

0.2 M HBTU Reagent Preparation

WARNING **CHEMICAL HAZARD.** ***HBTU can cause allergic reactions and skin irritations in sensitive persons. Wear protective clothing and gloves and prepare the HBTU solution in a properly vented fume hood.***

1. Pour the contents (40 mL) of the bottle labelled "HOBt/DMSO/NMP (0.2 M 1-hydroxybenzotriazole/Dimethyl sulfoxide/N-Methylpyrrolidone)" into the bottle labelled "Peptide Synthesis Reagent A, HBTU, 8 mmoles".
2. Tightly cap and invert the bottle to dissolve the HBTU.
3. Write the date of preparation on the HBTU bottle label. This prepared reagent can be used for up to six weeks.
4. Locate the delivery line for the HBTU. Press a reagent line filter onto the end of the delivery line. One reagent line filter can be used for five consecutive bottles, or approximately 200 mL, of HBTU reagent.

5. Place a bottle seal on the rim of the HBTU bottle.

   With the reagent delivery line inside, hold the bottle straight up and turn it carefully into the threads of the ratchet cap on Synergy (Figure 2-3). The bottle should fit firmly in place.

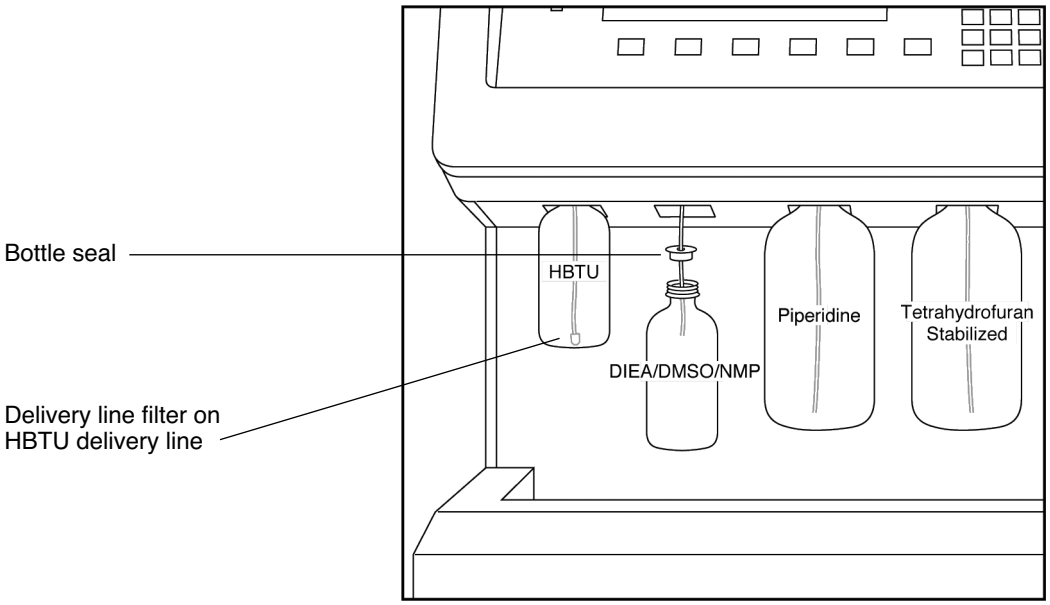


Figure 2-3. *Reagent bottle positions on the front of Synergy.*

6. Run Flow Test 8 to check HBTU delivery each time you replace the filter on the end of the HBTU delivery line. One delivery line filter can be used for five HBTU bottles or about 200 mL reagent.

Reagent bottle positions: When all the reagent bottles are in place, from left to right, facing the front of the synthesizer, the labeled reagent bottles should be HBTU in position A; DIEA/DMSO/NMP (0.4M N,N-diisopropylethylamine/dimethyl sulfoxide/N-methylpyrrolidone) in position B; Piperidine in position 1; and THF (Tetrahydrofuran) in position 2 (Figure 2-3). DMF (N-dimethylformamide) sits in a safety carrier on the side of the instrument, next to the waste bottle.

3. Check the waste bottle and empty, if necessary.

Waste generated per cycle: approximately 65 mL

The one-gallon (approx. 4 L) waste bottle accommodates approximately 62 synthesis cycles. Reagent usage is influenced by the conductivity produced during synthesis, so the waste generated varies according to the characteristics of the peptide sequence. Difficult sequences require longer deprotection and washing times and so generate more waste.

Multiply the number of amino acids in your synthesis by 65 mL to estimate how much waste will be generated. If the amount of waste generated is likely to exceed the space available in the waste bottle, replace the waste bottle with an empty bottle.

Synergy generates hazardous organic waste. Waste must be handled, stored, and disposed of in accordance with federal, state, provincial, and local hazardous waste regulations.

WARNING	**HAZARDOUS WASTE.** ***Waste produced by Synergy can be hazardous and can cause injury, illness or death. Handle all liquid, solid, and gaseous waste as potentially hazardous. During transfer, the waste container must be tightly sealed with the waste cap provided. Read all applicable Material Data Safety Sheets and the Synergy Waste Profile. Always wear gloves and handle hazardous waste in a fume hood that is connected in accordance with all installation requirements.***

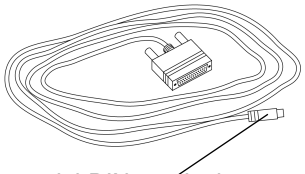
How to replace the full waste bottle

Note	*Use this procedure when the instrument is not running a synthesis. During a synthesis, see* ***How to remove and replace the full waste bottle during synthesis*** *on page 2-23.*

1. Unscrew the waste cap assembly on the waste bottle. You do not have to toggle the Pressure/Vent switch.
2. Tightly seal the waste bottle with the waste cap provided for transportation. Remove the waste bottle from the safety carrier and safely dispose of its contents.
3. Place an empty waste bottle in the safety carrier and screw the waste cap assembly onto the waste bottle.

4. Check the printer power, paper supply, and cable connections

When properly connected to Synergy and turned on, the HP ThinkJet™ Printer prints a conductivity trace of each cycle of deprotection and coupling that occurs during a synthesis.



- Verify that the mini-DIN terminal end of the RS 232 cable is plugged into the printer port of Synergy.
- Check the printer paper supply and replenish, if necessary.
- Turn on the printer power switch on the rear of the printer. The red light labeled “PWR” on top of the printer appears when the printer is turned on.
- If the yellow light next to the blue button labeled “Aa” is blinking, press the blue button to set the top line of the paper to the current line.

- Refer to ***Adjusting the Conductivity Trace Printout*** on page 4-4 for instructions on setting the printer speed, choosing the cycle number option, and adjusting the scale of the conductivity traces on the printout.

Note ***The "Computer" port above the Printer port currently has no user related functions. It may be used occasionally by trained Applied Biosystems Service personnel.***

Select and Load the Columns for Your Synthesis

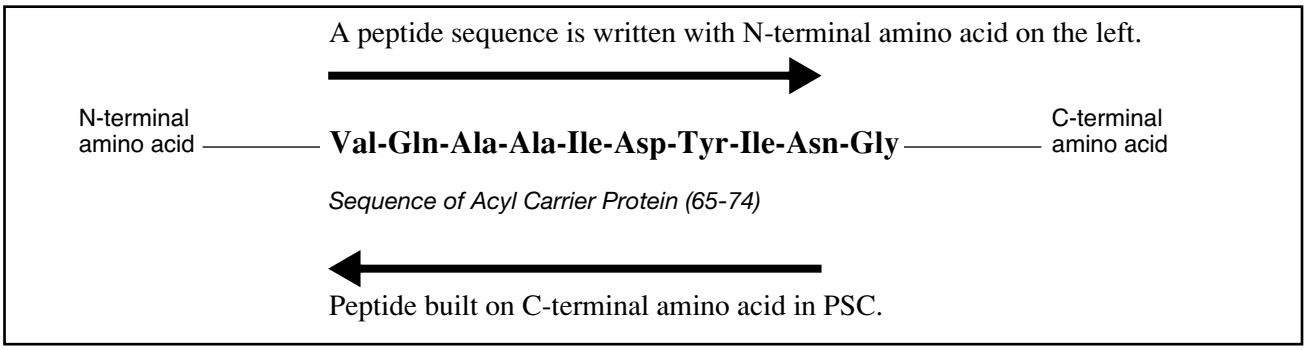
After you have completed all four of the synthesis preparation steps listed on page 2-5, Synergy is ready to perform a synthesis. Place one peptide synthesis column (PSC) in the peptide synthesis column position and the appropriate amino acid columns (AAC) on the amino acid column wheel. The wheel can hold up to 30 amino acid cartridges.

Note ***AACs and PSCs cannot be used interchangeably.***

1. Determine the sequence of the peptide to be synthesized.

   The labels on the AACs and PSCs display the amino acid abbreviations designated by the International Union of Pure and Applied Chemistry (IUPAC). Table 2-3 on page 2-13 lists these amino acid abbreviations.

   Peptide sequences are traditionally written with the N-terminal amino acid on the left and the C-terminal amino acid, or amide, on the right. Synergy synthesizes the peptide from the C-terminal position to the N-terminal position.



2. Place the PSC that corresponds to the C-terminal amino acid in the peptide synthesis column position (Table 2-4). PSCs that contain resin attached to amino acid are also called pre-loaded resins.

   If the peptide has an amide (NH_2) at the C-terminal position, place an amide resin PSC in the peptide synthesis column position. Amide resin PSCs are labeled with the abbreviation "AM."

Caution ***Leaks in the peptide synthesis column position can damage Synergy. To assure a leak-proof connection between the peptide synthesis column and the luer fittings, push the luer fittings onto both column ends as tightly as possible. Be careful not to twist the column.***

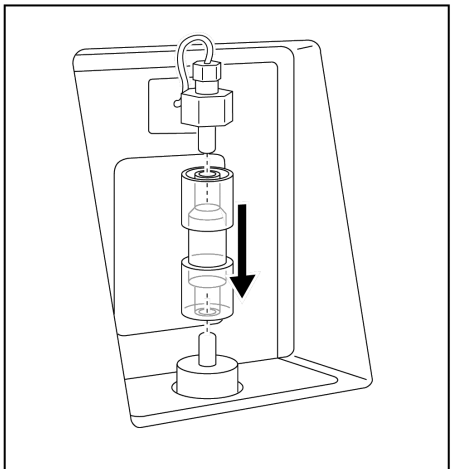


Figure 2-4. *Place the PSC in the peptide synthesis position*

3. Place the AACs on the wheel.

   If you used a pre-loaded resin in the PSC, the AAC for the second amino acid from the C-terminal goes in position 1. Ifyou used an amide resin, the AAC for the C-terminal amino acid goes in position 1. Place the N-terminal AAC on the wheel last, after all the other amino acids in the sequence are in place. **Do not leave any positions empty between the AAC in position 1 and the N-terminal AAC.**

With the pre-programmed lines, you do not have to tell Synergy how many amino acids are in the peptide. Instead, when the sensors on the autosampler detect a position on the wheel without an AAC, they signal the controller to end the synthesis. Therefore, do not leave any empty positions on the wheel, except after the N-terminal AAC.

IMPORTANT *The order of the amino acid columns on the amino acid column wheel determines the sequence of amino acids in the peptide.*

For example, to synthesize Acyl Carrier Protein (65-74), shown on page 2-10, place the G/Gly peptide synthesis column in the PSC position.

Place an N/Asn amino acid column in position 1 -on the amino acid column wheel. Place an I/Ile amino acid column in position 2. Place a Y/Tyr AAC in position 3. Place an D/Asp in position 4, place another I/Ile position 5, place A/Ala in positions 6 and 7, place Q/Gln in position 8.

The N-terminal amino acid, V/Val, goes on last, in position 9.

Figure 2-5 illustrates how, when the AACs are in the correct order, you can hold up the wheel and read the peptide sequence with the N-terminal AAC on the left and the missing C-terminal amino acid in the PSC, if the PSC contains a pre-loaded resin.

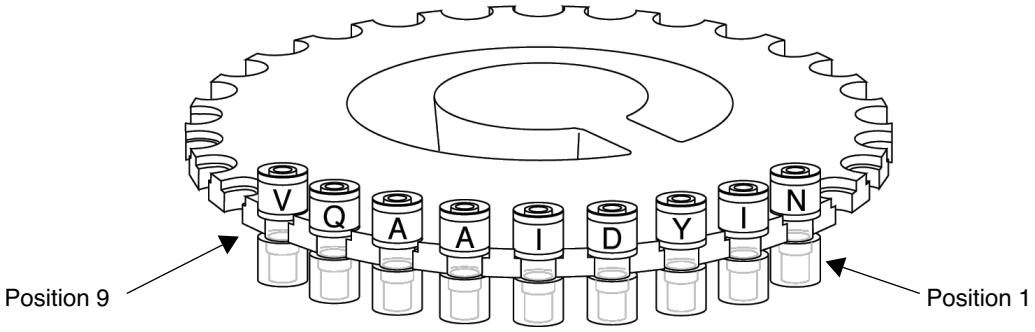


Figure 2-5. *AACs in positions on the wheel to synthesize ACP (65-74)*

Note	***After a synthesis, the AACs still contain white, inert material, but very little amino acid remains. AACs contain only enough amino acid for one synthesis cycle. They cannot be re-used.***

A few words about amino acid (AAC) and peptide synthesis (PSC) columns

Applied Biosystems AACs and pre-loaded PSCs both contain amino acid and polymeric material. The amino acid in the AACs is associated temporarily to an inert polymer. When the amino acid is removed from the AAC during synthesis, the inert polymer stays in the column. In the PSCs, the amino acid is bound to a lightly cross-linked, polystyrene resin. The bond between the amino acid and resin in the PSC is not broken until after synthesis is complete, during the TFA cleavage procedure described in Chapter 3.

IMPORTANT	***AACs and PSCs cannot be used interchangeably. AACs have a light blue label; PSCs have a completely white label.***

These columns are designed to be used one time only; once a column has been opened, its leakproof seal is broken. Treat these used columns as a solid hazardous waste and dispose of them with other disposable equipment that has been in contact with chemicals.

Caution	***Re-using PSCs or AACs can cause reagent leakage on the conductivity cell or the autosampler jaw seals and damage to Synergy. Discard these columns after one use.***

Unused AACs and PSCs can be safely stored at room temperature for up to one year after the date of shipment.

WARNING **SOLID HAZARDOUS WASTE** ***Used AACs and PSCs have been exposed to DMF (N, N-dimethyl formamide), THF (tetrehydrofuran), and other hazardous chemicals. Dispose of used columns in accordance with local, state, and federal regulations regarding solid hazardous waste. See the Synergy Waste Profile in Chapter 1 of this User's Manual.***

Table 2-3. Amino acid abbreviations

AminoAcid	3-letter code	1-letter code
alanine	Ala	A
arginine	Arg	R
asparagin	Asn	N
aspartic acid	Asp	D
cysteine	Cys	C
glutamine	Gln	Q
glutamic acid	Glu	E
glycine	Gly	G
histidine	His	H
isoleucine	Ile	I
leucine	Leu	L
lysine	Lys	K
methionine	Met	M
phenylalanine	Phe	F
proline	Pro	P
serine	Ser	S
threonine	Thr	T
tryptophan	Trp	W
tyrosine	Tyr	Y
valine	Val	V

Check the Run File

The three pre-programmed lines in combination can perform most routine syntheses. Chapter 6, ***Advanced Operations***, describes how and when to modify the Run File.

IMPORTANT *Edited Run lines remain part of the Run File until you return to the Run Editor and erase them.*

If you have never used the Run Editor Menu to modify the Run File, the Run File contains the pre-programmed lines shown in Table 2-1 on page 2-5.

If you have modified the Run File, check that it contains the appropriate Run lines for the synthesis you are about to start.

Start the Automated Synthesis on Synergy

When all the appropriate columns are in place and the amino acid column wheel is on the instrument, you can begin the automated synthesis from the Run Control Menu.

Synthesis Startup Checklist

1. Check the external gas supply	_____
2. Checks level of 5 reagent bottles	_____
3. Check waste bottle, empty full waste bottle	_____
4. Check printer power, paper supply, and cable connections	_____
5. Select and load PSC and AACs (C-terminal -> N-terminal)	_____
6. Check the Run File	_____
7. Check Run Control Menu settings	_____

How to start synthesis in the Run Control Menu

1. Press the ***run control*** soft key in the Main Menu to go to the Run Control Menu (Figure 2-6).
2. **To direct Synergy to use the lines that are currently in the Run File,** the word **YES** should appear after "Run Begin" and after "Run End" on the Run Control Menu.

   If you want the printer to generate a conductivity trace of the synthesis, the word **YES** should appear after the word "Print:" on the Run Control Menu.

   To change any entry on the Run Control Menu, use the arrow keys to move the cursor to the right or left. Press the ***YES*** or ***NO*** soft key to change the entry.
3. Press the ***START*** soft key in the Run Control Menu to begin synthesis. The Run Monitor appears as synthesis begins (Figure 2-6).

   Synergy's controller checks for pressure leaks in the PSC fittings during the first few minutes of synthesis. Visually check around the PSC luer fittings for leaks. If you detect a leak at either end of the PSC, push and twist the end into its luer fitting.

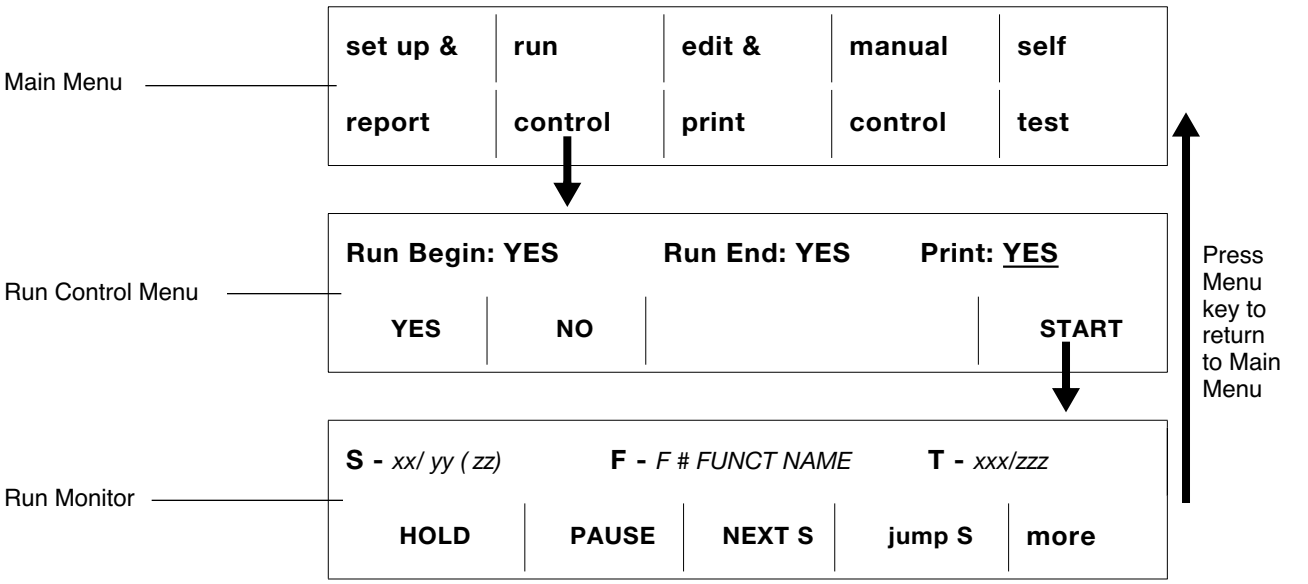


Figure 2-6. *How to go to the Run Control Menu*

Occasionally, small droplets may form around the middle of the PSC. Use a tissue to wick away small amounts of liquid. If the PSC continues to leak, terminate the synthesis (see ***How to terminate a synthesis*** on page 7-11) and restart the synthesis with a new PSC. If no leaks are detected in these initial minutes of operation, you may leave the instrument unattended during synthesis.

Run ended: *13:58*	**Run began:** *10:54*
Cycles run: *3*	**OK**

4. This display appears when the synthesis is completed. Press the ***OK*** soft key. If the printer is on, this record of the synthesis appears at the end of the conductivity trace. See ***Interpreting the conductivity trace*** on page 2-16 for a description of the features of a conductivity trace.

Note ***After the synthesis, the AACs still contain white, inert material, but very little amino acid remains. AACs contain only enough amino acid for one synthesis cycle. They cannot be re-used.***

5. Verify the sequence of amino acids in the peptide sequence before removing and discarding the AACs on the wheel. Record the sequence on the conductivity trace printout or in your laboratory log book.

Interpreting the conductivity trace

Compare the trace your instrument has generated to the conductivity trace of LAGV shown in Figure 2-7. Note that there are actually two overlapping conductivity traces that reflect two conductivity ranges. Trace Features 1-4 and Trace Feature 7 are in the low conductivity range; Trace Features 5 and 6 are in the high conductivity range.

Typically, at the beginning of every trace, you should observe a series of upward spikes as the resin in the PSC solvates before the DMF baseline is established. Following resin solvation, each cycle consists of a deprotection peak (Trace Feature 1) followed by a coupling plateau (Trace Features 5 and 6). Your trace should have one cycle for each AAC on the wheel. At the end of the synthesis, the trace should show one more deprotection followed by a drop below the DMF baseline when THF washed through the PSC.

Seven features of the trace your instrument has generated should closely match the trace shown in Figure 2-7. These seven Trace Features should be present in each complete cycle of deprotection and coupling.

1. **Beginning deprotection:** a single, sharp peak
2. **Deprotection complete:** a level horizontal line slightly higher than the DMF baseline
3. **Peperidine washout:** a return to DMF baseline.
4. **Amino acid activation:** a level baseline consistent with point 4
5. **Coupling begins:** a rapid rise followed by a steady, high plateau
6. **Pump/AAC wash:** a level consistent with point 5
7. **Coupling solution washout:** a drop that returns to DMF baseline

For descriptions of irregular traces, see ***Irregular Conductivity Traces*** on page 7-3.

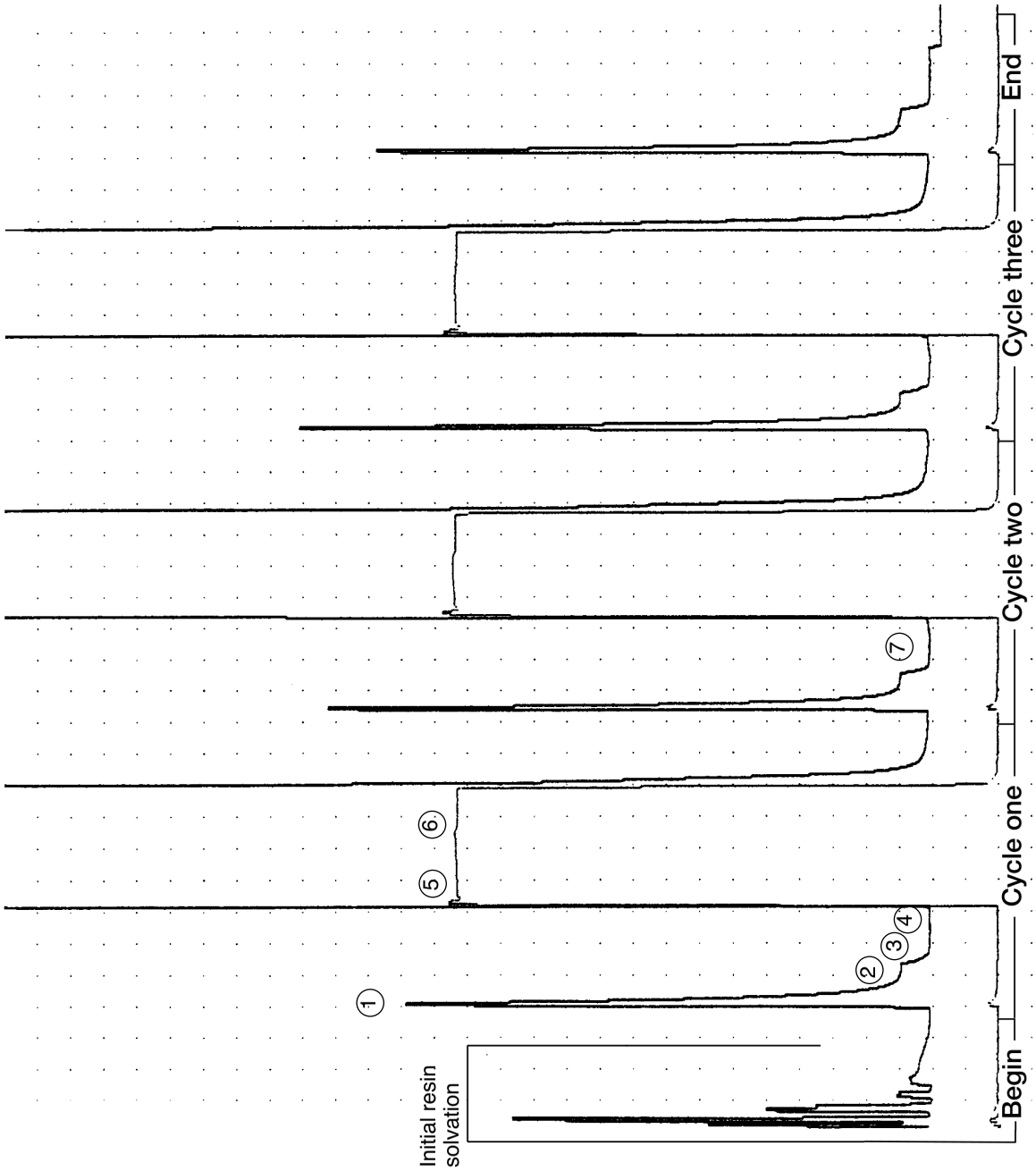


Figure 2-7. *Sample Synergy conductivity trace (scaled to fit page)*

Synthesis Interruptions

If you are using the pre-programmed lines, the synthesis normally progresses from start to finish without any need for user interaction with the instrument. However, when a pressure test fails or a mechanical error is detected, Synergy temporarily interrupts synthesis.

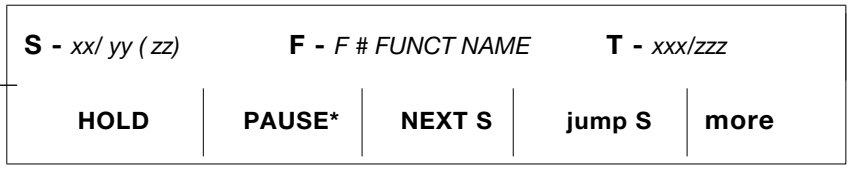
At other times, you may have to interrupt synthesis to fix a problem that needs immediate attention, such as an empty reagent bottle, a full waste bottle, a power failure, or a printer jam.

Pressure Test Failures and Error Messages during syntheses

Synergy automatically interrupts a synthesis whenever it detects a leak or a mechanical error.

If a pressure test fails during a synthesis, the LCD displays the following message: Pressure test failed:

Press the ***OK*** soft key to return to the Run Monitor when a pressure test fails.



After a pressure test failure, the Run Monitor displays an asterisk (*) after the word ***PAUSE***, to indicate that Synergy has automatically suspended operation. If you press the ***PAUSE**** soft key to resume operation without first finding the leak in the pressure system, the instrument may continue to fail the pressure test. Refer to ***What to do after a pressure test failure*** on page 7-12 for further instructions.

Mechanical failures that cause an interruption in the synthesis can occur when the autosampler jaws do not completely open or close, or when the amino acid column wheel does not move properly. If the optical sensors do not detect a column in the jaws, synthesis ends.

When a mechanical failure interrupts synthesis, the LCD displays an error message on the top line. Make a note of this error message and refer to ***Error Messages*** on page 7-20. Press the ***OK*** soft key to go to the Run Monitor when an error message appears.

Operator Interruptions to Synthesis

IMPORTANT ***The procedures described in this section should be used only in emergencies and not on a routine basis. Before each synthesis, follow the steps described at the beginning of this chapter to prevent unnecessary interruptions to synthesis.***

When a problem requires immediate attention you can interrupt synthesis in the Run Monitor. However, before you interrupt a synthesis, you should determine what module, or stage in the process, the instrument is performing and how an interruption at that stage might affect the final product. Then you can determine at what future module you can program the instrument to interrupt synthesis without adverse affects on the final product. Table 2-4 lists the module names and the operation associated with that module.

Table 2-4. Pre-programmed Instrument Modules and Operations

Module	Operation
a	**A**ctivation
b	**B**egin synthesis
c	**C**oupling
d	**D**eprotection
e	**E**nd synthesis
f	reagents **F**low through valve blocks and PSC
g	**G**urgle-solvents wash AAC and transfer vessels
h	alternate to module a, for non-standard amino acids
i	**I**ncrement-moves the wheel to the next AAC position
j	**J**aws close on AAC and pressure test

How to determine the cycle and module Synergy is currently running

1. Press the ***more*** soft key in the Run Monitor to make the second Run Monitor appear. The ***set int*** soft key should be visible.

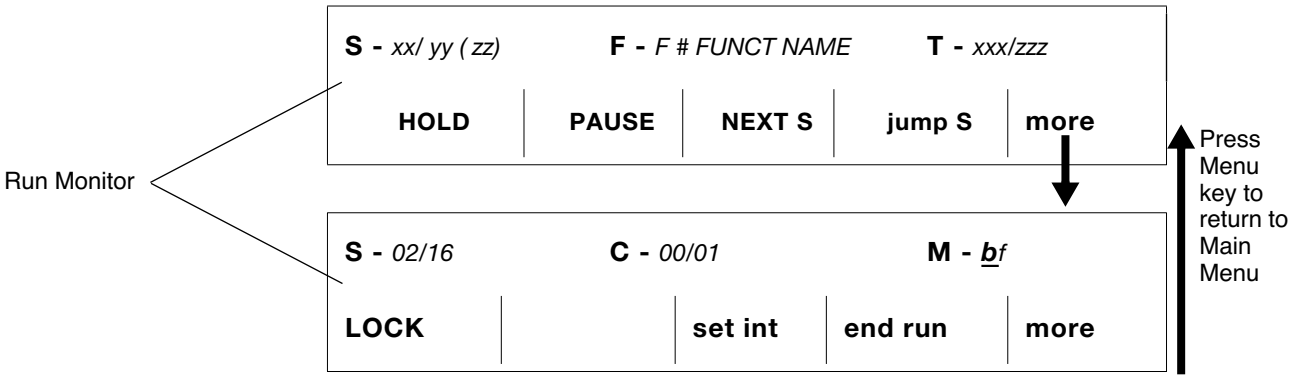


Figure 2-8. *How to determine the cycle and module currently running*

2. Read the top line of the second Run Monitor display to determine which module the instrument is currently running. The abbreviations in the top line indicate, from left to right:

- S (Step)—the current step number/total number steps in this module.
- C (Cycle)—the current cycle number/total number of cycles in this synthesis. The begin cycle is always cycle 0 (zero). With pre-programmed lines, the value for the total number of cycles increases with each complete cycle, until the END line is run.
- M (Module)—the cursor blinks under the module that is running.

For example, in the second Run Monitor shown in Figure 2-8, the instrument is on the second step of 16, in Module b, and in the begin cycle of the synthesis.

Once you know the module that is running, refer to Table 2-5 to determine the best place to set an interruption.

Table 2-5. Action Items and Response During Synthesis

Action Item	Response
Replace empty reagent bottle*	***set int*** at Module g, j, or i, step 1 (one)
Replace external gas tank**	***set int*** at Module g, j, or i, step 1 (one)
Remove full waste bottle***	***set int*** at Module c, step 1 (one)
Powerfailure	Continue synthesis, unless failure occurred during module a or h and lasted more than 2 minutes
Replace printer paper	Go to Setup & Report Menu to cancel print

* Piperidine may be changed at any module **except Module d** and requires no system depressurization.

** Change external gas tank when pressure drops below 300 psi.

***It waste is backing up into external waste ventilation, **IMMEDIATELY PRESS THE *PAUSE* soft key and empty the waste bottle**.

How to set an interruption to synthesis

1. Follow the directions on page 2-19 to determine what cycle and module Synergy is currently running.
2. Consult Table 2-5 to determine where to interrupt synthesis. Choose the first possible module in the current or next cycle.

   For example, if you need to change the HBTU bottle and Synergy is running Cycle 1, Module c, set the interrupt to occur at Cycle 1 (one), Module g.
3. Press the ***set int*** soft key on the Run Monitor to go to the first Set Interrupt Menu.

   The first Set Interrupt Menu displays the modules that make up "Line 1" in the pre-programmed set of lines. Most interruptions should occur in one of these modules.

   When the first Set Interrupt Menu appears, the cursor is under the Cycle number (Figure 2-9).

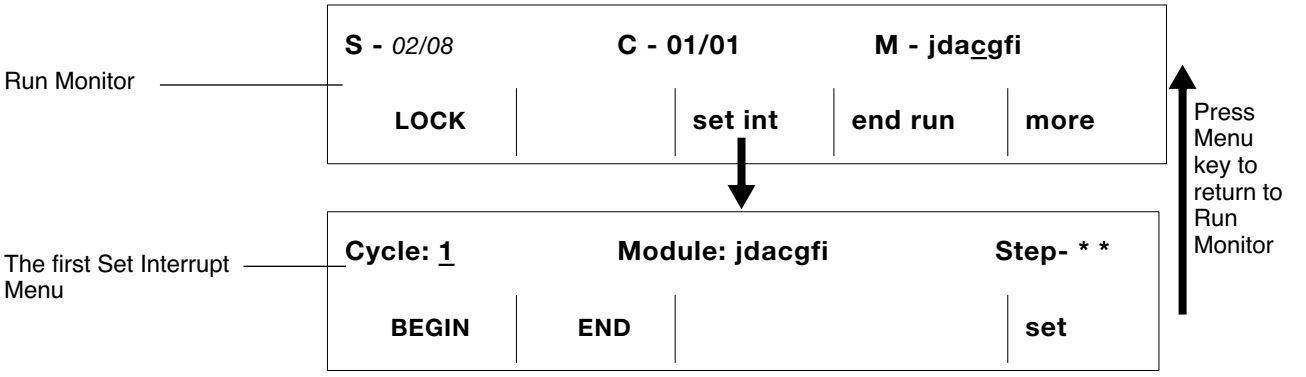


Figure 2-9. *How to set the interrupt Cycle number*

4. Use the numeric keys to enter the Cycle number where the interruption should occur.
5. Use the arrow key to move the cursor under the appropriate module letter. Press the ***set*** soft key and the Set Interrupt Step Menu appears (Figure 2-10).

   The top line in the Set Interrupt Step Menu displays the Module letter you have just designated. The cursor appears after the letter "S" under step number 1 (one). Most interruptions should occur in Step one.

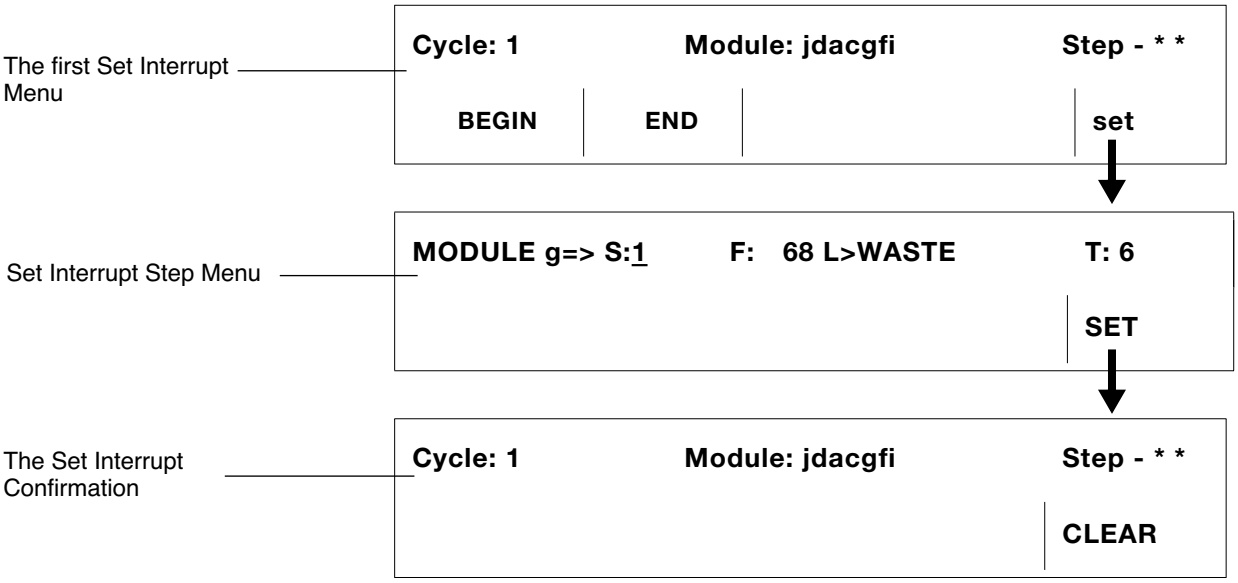


Figure 2-10. *How to set the interrupt Step number*

6. With Step 1 selected in the Set Interrupt Step Menu, press the ***SET*** soft key to complete the Set Interruption. The top line of the next LCD should confirm the

Cycle number, Module, and Step number of the interruption setting.

The Cycle number of the interruption appears after the word Cycle, the cursor appears under the Module letter, the Step number appears after the word Step.

To change the interruption setting, press the ***CLEAR*** soft key and repeat this procedure, starting on page 2-20.

7. To return to the Run Monitor, press the Menu key. When synthesis is interrupted the ***PAUSE**** soft key appears on the Run Monitor.

How to replace nearly empty reagent bottles during synthesis

IMPORTANT *If, during a synthesis, you notice that a reagent bottle on Synergy is completely empty and the conductivity trace is unusual, the synthesized peptide will probably be inferior. It is more efficient to end the synthesis and start over with full reagent bottles and fresh columns.*

1. **If the HBTU, DIEA, THF, or DMF bottles are almost empty**, follow ***How to set an interruption to synthesis*** on page 2-20. Set the interruption at step 1 of either Module g, j, or i. When synthesis is interrupted the ***PAUSE**** soft key appears on the Run Monitor.

   If the piperidine bottle is almost empty, you do not need to depressurize the system to change the bottle. A soft hiss of pressurized gas escapes when you unscrew the ratchet cap. **Do not change the piperidine bottle when Module d is running.**

2. When Synergy interrupts synthesis, switch the Pressure/Vent switch to Vent. Wait 30 seconds for the internal pressure to drop to zero.
3. Remove all the empty reagent bottles at once and replace them with full bottles. Refer to Step 4. of the ***Bottle Changing Procedure*** on page 4-9 for a thorough description of this process.
4. When the full reagent bottles have been placed on Synergy, switch the Pressure/Vent switch to Pressure and wait 60 seconds for the internal pressure to stabilize.
5. Press the ***PAUSE**** soft key to resume synthesis.

   With this procedure, you change reagent bottles without going through the standard bottle change procedure described in Chapter 4. The Synergy controller does not check the replaced reagent bottles for leaks until synthesis progresses to module j. If a pressure test fails in module j after you have replaced reagent bottles, it could indicate a bad bottle seal, a crack in the rim of the bottle, or a bottle has not been tightly screwed in place.

How to replace an empty external gas tank during synthesis

1. Follow the procedure ***How to set an interruption to synthesis*** on page 2-20. Set the interruption to occur at step 1 of either Module g, j, or i.
2. When the ***PAUSE**** soft key appears on the Run Monitor, follow the procedure ***How to change the external gas tank*** on page 2-6.
3. When the gas tank has been replaced, press the ***PAUSE**** soft key to resume synthesis.

How to remove and replace the full waste bottle during synthesis

Caution	***It the liquid waste overflows into the external waste ventilation system during a synthesis, IMMEDIATELY PRESS THE PAUSE SOFT KEY TO INTERRUPT SYNTHESIS. DO NOT WAIT FOR MODULE c.***

1. If the waste bottle is almost full, follow the procedure ***How to set an interruption to synthesis*** on page 2-20 and set the interruption to step 1 of Module c.
2. When the ***PAUSE**** soft key appears, follow the procedure ***How to replace the full waste bottle*** on page 2-9.
3. When the empty waste bottle is in place, press the ***PAUSE**** soft key to resume synthesis.

How to replace or re-align printer paper during synthesis

If the printer runs out of paper, or if the paper is jammed in the printer, you can retrieve the conductivity data from the buffer, or memory, for the run. The buffer holds all the conductivity data for one peptide synthesis only. Each time a new synthesis begins, old data is erased to clear the buffer. Once the data is erased, you cannot retrieve it or print it.

With the procedure described here, you interrupt data printing first and then replace or re-align the printer paper.

1. Press the Menu key to return to the Main Menu.
2. In the Main Menu, press the ***set up & report*** soft key, then press the ***print reports*** soft key to go to the Print Reports Menu (Figure 2-11).
3. Press the ***CANCEL PRINT*** soft key to stop sending conductivity data to the printer.
4. Replace or re-align the printer paper. Refer to the ThinkJet *Owner's Manual* for instructions.

5. Press the ***MONITOR DATA*** soft key to resume printing. The printer will now start printing data from the beginning of the current synthesis. It takes from 7 - 15 minutes per cycle to reprint data, depending on the chart speed set in the Chart Recorder Menu. (See ***How to change the chart speed*** on page 4-5).

Note *Press the* **MONITOR DATA** *soft key to print the conductivity data for the most recent run if Synergy is not currently synthesizing a peptide (Figure 2-11).*

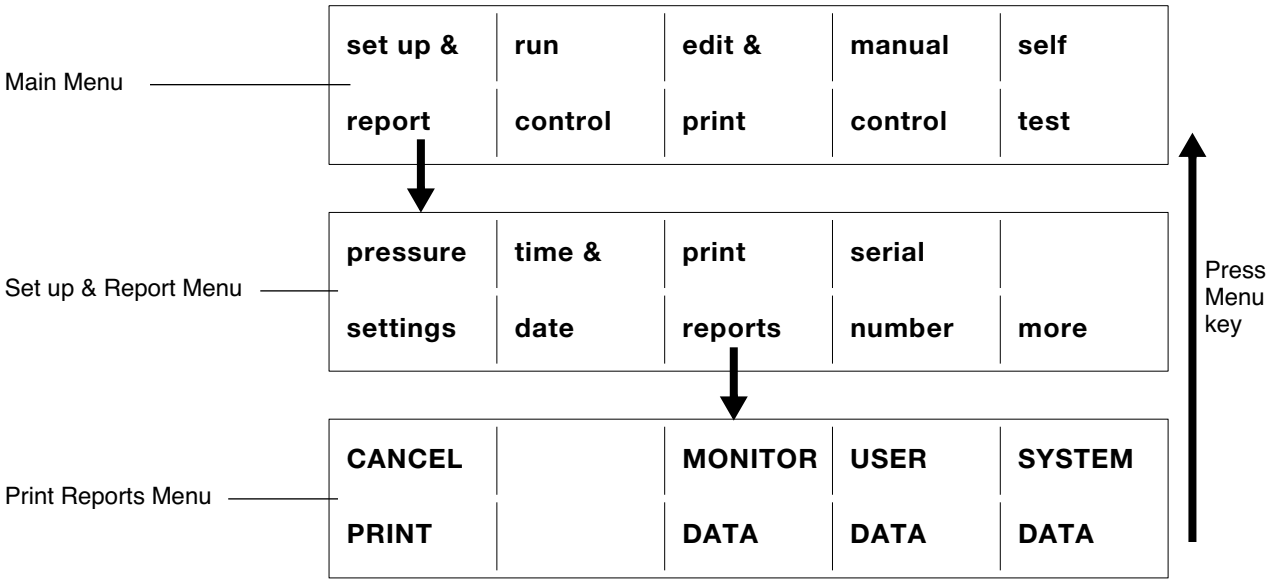


Figure 2-11. *How to go to the Print Reports Menu*

Powerfailures

Whenever a powerfailure occurs, the autosampler jaws open automatically. When power returns, the wheel automatically moves to home position so that position 1 on the wheel is between the jaws. If you were running a synthesis before the powerfailure, when power returns the jaws close on the column in position l to check the wheel alignment. If the wheel is aligned correctly, the jaws open and the wheel returns to the position it was in before the powerfailure occurred.

Note ***If the printer is running when a powerfailure occurs, you must realign the top of the paper in the HP PaintJet when power returns. Follow the instructions in the ThinkJet Owner's Manual.***

When power first resumes after a powerfailure, Synergy displays the message: ***There was a flower failure while the synthesizer was running.*** Press the ***OK*** soft key after this message, and Synergy displays the Main Menu.

If a synthesis was running before the powerfailure occurred, the synthesis continues until the controller encounters Function 9, P/F PAUSE. If the length of the powerfailure exceeds pre-determined limits, Synergy displays the message: ***The power was off too long to resume the run.***

When an interruption occurs during synthesis, you have to decide whether or not to continue synthesis. Your decision should be based on the following subjective criteria: how urgently you need the peptide; how much you have already invested in time and energy; how much more you are willing to invest to synthesize this peptide.

During modules a and h, the activation modules, amino acids are rapidly activated. For optimal chemistry results, coupling should occur immediately after activation, especially after activation of arginine. The greater the amount of time that passes before activated amino acids come in contact with the deprotected amine, the greater the chances that side-chain reactions will occur.

If a powerfailure occurs in any module except modules a and h, the pre-programmed lines allow a powerfailure of up to 60 minutes. If power resumes within 60 minutes of the failure, Synergy will continue the synthesis. If the powerfailure lasts longer than 60 minutes, Synergy will interrupt the synthesis when the controller encounters Function 9, P/F Pause.

If a powerfailure occurs during module a or h, Synergy tolerates an outage of only 2 minutes or less. If power resumes within 2 minutes of the failure, Synergy will continue the synthesis. If the powerfailure lasts longer than 2 minutes, Synergywill interrupt the synthesis when the controller encounters Function 9, P/F Pause.

After a powerfailure in any module except module a or h, you can press the ***PAUSE**** soft key in the Run Monitor to continue synthesis with little risk of jeopardizing the condition of the peptide. If a powerfailure occurs during module a or h and lasts longer than 2 minutes, it may be more cost-effective to end the synthesis and start over from the beginning with fresh AACs and PSC. If you prefer to continue the syn-

thesis after along powerfailure occurs in module a or h, the following procedure describes how to recover from the powerfailure.

How to recover from a long powerfailure in module a or h

In this procedure, any activated amino acid that may be left in the instrument lines or transfer vessels after the powerfailure is first washed out to the waste bottle. Then you end the synthesis run, re-arrange the AACs on the wheel, and continue the synthesis.

Note	***If you continue synthesis after a powerfailure that was longer than 2 minutes occurred in module a or h, you have to replace the AAC for the amino acid that was being activated when the powerfailure occurred.***

After a powerfailure in module a or h, the synthesizer interrupts synthesis at Function 9, P/F Pause. The Run Monitor displays the ***PAUSE**** soft key.

1. In the Run Monitor, press the ***jump S*** soft key (Figure 2-12).

   The Jump Step Menu displays the module, step, and function of the synthesis interruption. This is **not** necessarily the same step and function where the powerfailure occurred.

2. In the Jump Step Menu, press the ***module*** soft key to go to the Jump Module Menu.

3. In the Jump Module Menu, use the arrow key to move the cursor under module g. Press the ***JUMP*** soft key twice to return to the Run Monitor.

4. Set an interruption to occur at Step 16 of module g. (See Steps 3- 7 of ***How to set an interruption to synthesis*** on page 2-20.)

5. In the Run Monitor, press the ***PAUSE**** soft key to start running module g.

   The first 9 steps of module g wash out the activated amino acid that was left in Synergy after the powerfailure. The last 7 steps of module g gas-dry the Synergy “pump”.

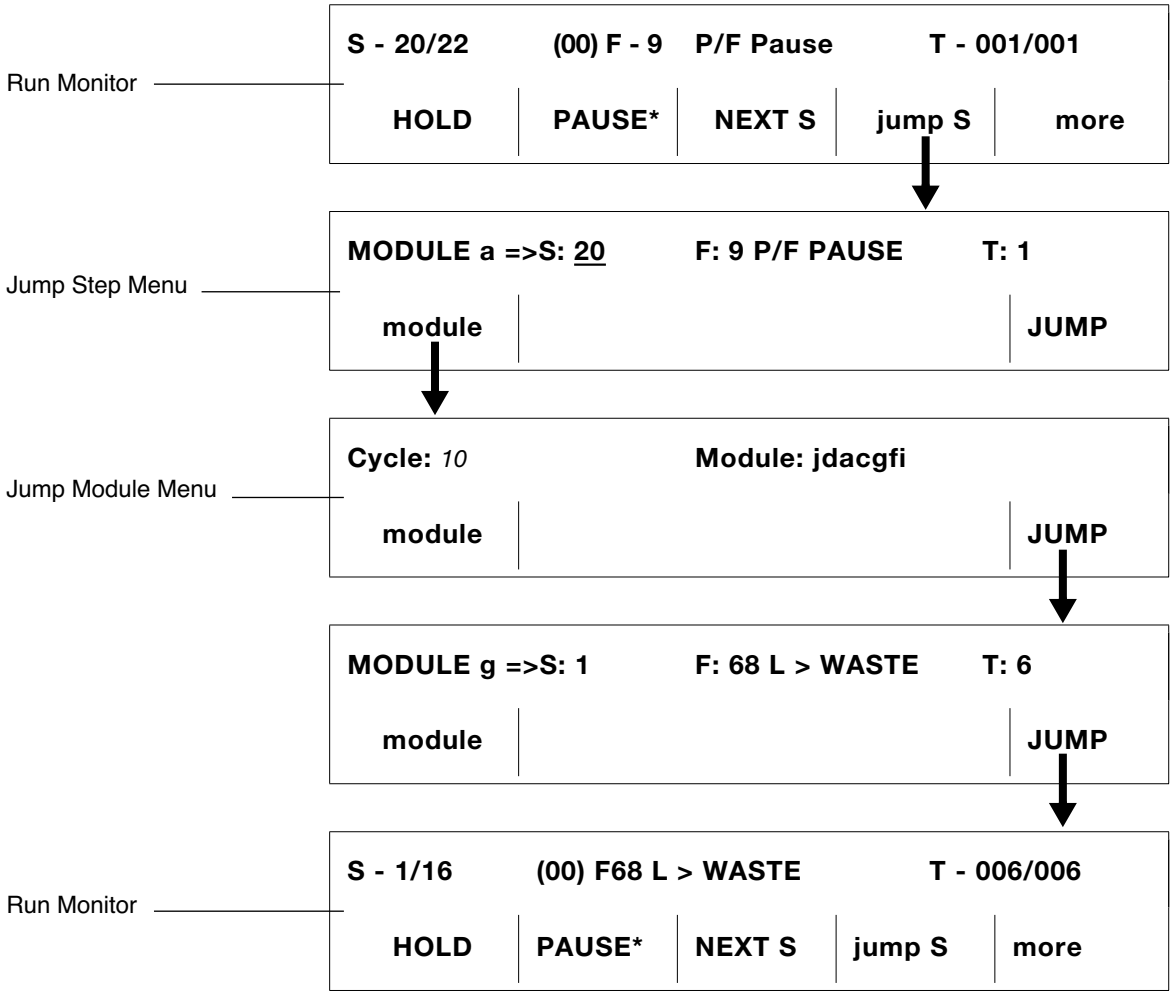


Figure 2-12. *How to jump out of module a after a powerfailure*

6. When synthesis is interrupted at Step 16 in module g, end the run.
   a. In the Run Monitor, press the ***more*** soft key until the ***end run*** soft key appears (Figure 2-13).
   b. Press the ***end run*** soft key. When the message "Are you sure you want to end the run?" appears, press the ***END RUN*** soft key.

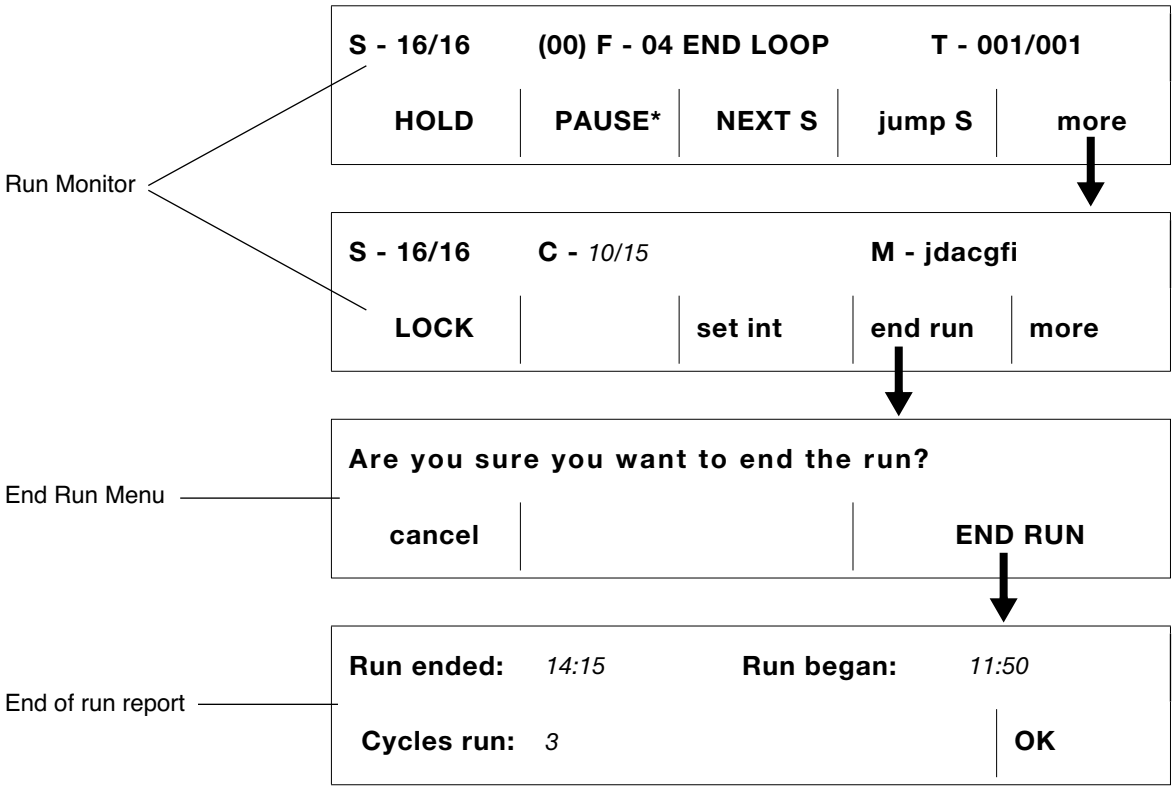


Figure 2-13. *How to end the run after a powerfailure in module a*

The LCD displays the time the run began and ended. The autosampler jaws are closed on the AAC that contained the amino acid that was being activated when the powerfailure occurred. In the steps that follow, you will open the autosampler jaws to free the wheel and re-arrange the AACs for the continuation of the synthesis.

7. Press the Menu key to go to the Main Menu, then press the ***manual control*** soft key to go to the Manual Control Menu.
8. Press the ***function*** soft key to go to the Function Menu (Figure 2-14).
9. Use the numeric keys to enter Function 53, OPEN JAWS. Press the ***ON*** soft key.

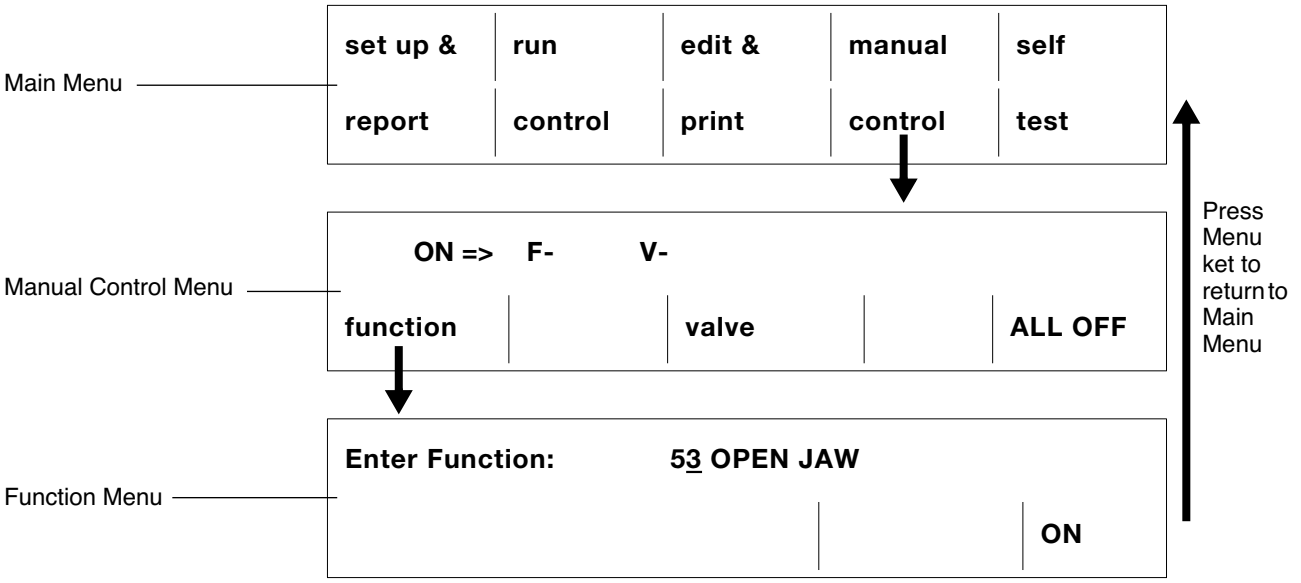


Figure 2-14. *How to manually activate the OPEN JAWS function.*

10. When the jaws open, remove the amino acid column wheel. Remove all the used AACs, including the column that contained activated amino acid when the powerfailure occurred.

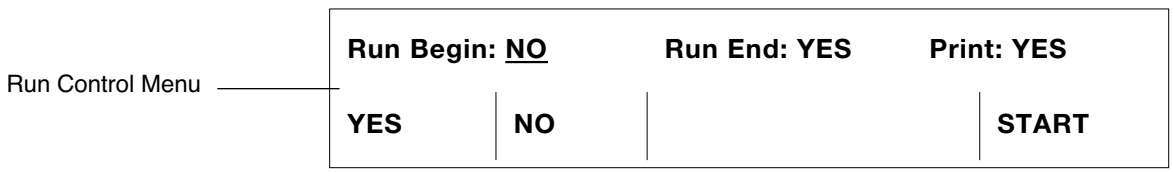
    During the first 5 steps of this procedure, the amino acid that was being activated when the powerfailure occurred was washed out of the Synergy "pump" to prevent its coupling to the peptide.

11. Replace the column that contained activated amino acid when the powerfailure occurred with a fresh, unused AAC. Place this AAC in position 1 on the wheel. Place the rest of the unused AACs on the wheel in the positions after position 1. There should be no empty positions between position 1 and the N-terminal AAC.

12. Replace the wheel.

13. Go to the Run Editor Menu and edit line 1 to read: j a (*or h*) c c c g f i. Add a second line with the standard cycle line, j d a c g f i, for the cycles that follow.

14. Press the Menu key to return to the Main Menu. In the Main Menu, press the ***run control*** soft key to go to the Run Control Menu.



15. In the Run Control Menu, move the cursor to the space after "Run Begin:" and press the ***No*** soft key. Then press the ***START*** soft key to continue the synthesis.

Synergy Shutdown Procedure

If Synergy is used regularly, there is no need to turn off the power or remove reagent bottles between syntheses. Its stand-by power consumption is equivalent to that of a 20-watt light bulb.

However, if Synergy will not be used for a month or longer, flush the reagent lines with DMF and nitrogen to prevent the lines from becoming blocked with reagent crystals. To perform this procedure, you need the four empty bottles that were shipped on Synergy, the plastic cap for the Waste port and the loop that connected the Pressure and Delivery ports at the beginning of the Installation Procedure.

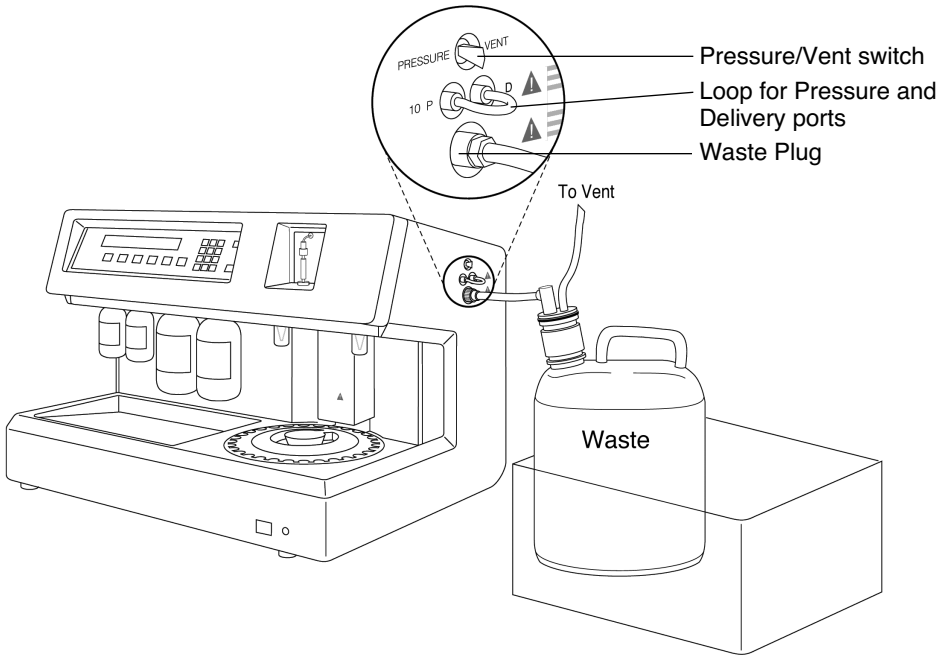


Figure 2-15. *Waste plug and loop for Pressure and Delivery ports*

WARNING **CHEMICAL HAZARD.** ***Some chemicals used with this instrument can be hazardous and cause injury, illness, or death. Wear protective eye glasses, gloves, and a laboratory coat when working with these reagents. To minimize exposure to and inhalation of these chemicals, cap and store the reagent bottles according to instructions found in the MSDSs.***

1. Pour DMF into two empty 40-mL bottles and one empty 200-mL bottle, so that the approximate depth of reagent in each bottle is 1 cm.
2. Switch the Pressure/Vent switch to Vent and wait 30 seconds for the internal pressure to drop.

3. Remove all the reagent bottles currently in position on the front of Synergy, with the following additional steps:
   - Disconnect the DMF bottle lines at the Pressure and Delivery ports. Remove the DMF pressure line first, then remove the DMF delivery line. Replace these lines with the connecting loop that connected the Pressure and Delivery ports during Synergy shipment (Figure 2-15). Remove the cap assembly from the DMF bottle; cap and seal the bottle tightly.
   - Replace the HBTU and DIEA bottles with the 40-mL bottles of DMF from step 1. Replace the piperidine bottle with the 200-mL bottle of DMF from step 1. Replace the THF bottle with an empty 200-mL bottle.
4. Switch the Pressure/Vent switch to Pressure.
5. In the Main Menu, press the ***self test*** soft key to go to the Self Test Menu. Press the ***bottle*** soft key in the Self Test Menu. Then press the ***prime*** soft key.

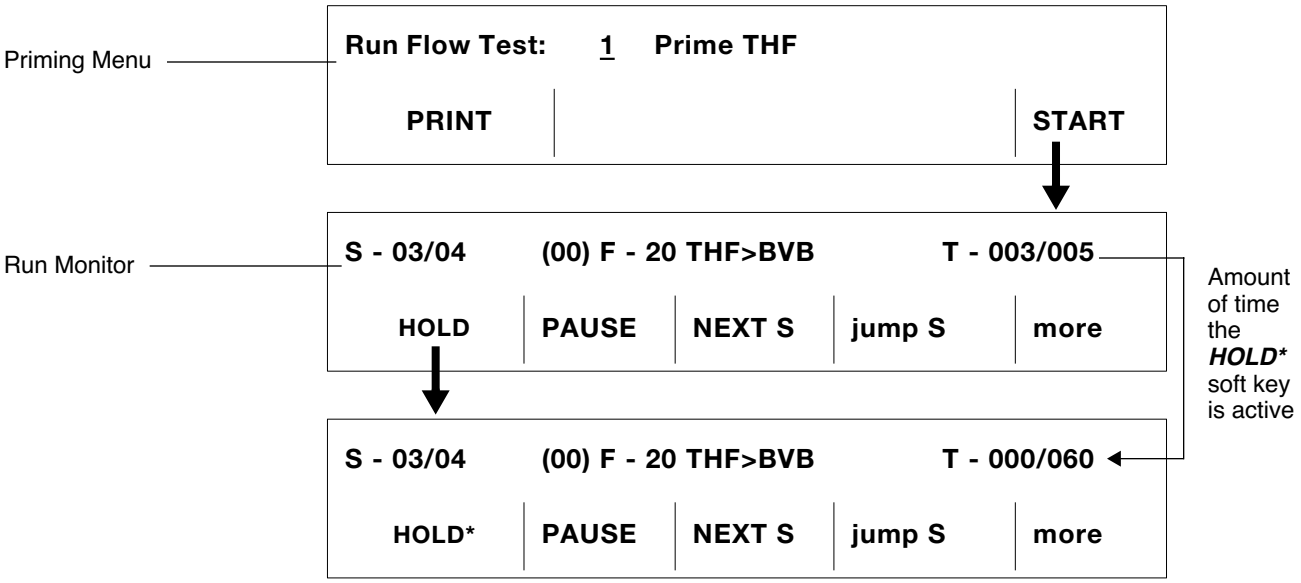


Figure 2-16. *How to hold the THF prime at step 3*

6. To begin a flow test, press the ***START***soft key. Observe the step number in the left corner of the Run Monitor. With all the reagent primes, **except the PIP (piperidine) prime**, press the ***HOLD*** soft key when the prime is in step 3. **With the piperidine prime**, press the ***HOLD*** soft key when the prime is in step 4. When one prime is finished, press the key labeled *"Next"* to proceed to the next prime.
   - During both the THF and DMF primes, hold the prime at step 3 for one minute to flush nitrogen gas through the reagent line. When the values after the T are 000/060, press the ***HOLD**** soft key to release the hold.

- During both the HBTU and DIEA primes, hold the prime at step 3 until all DMF in the reagent bottles has been delivered, then hold the prime for 60 more seconds to flush nitrogen through the reagent lines. Press the ***HOLD**** soft key to release the hold.
- During the PIP prime, hold the prime at step 4 until all DMF is drained from the reagent bottle, then hold the prime 60 seconds more. Press the ***HOLD**** soft key to release the hold and finish the prime.

  When the ***HOLD*** soft key is active, an asterisk (*) appears after the soft key name. As long as it is active, the software does not progress to the next step and Synergy continues to perform the step being held. The values that appear after the letter T count the number of seconds the step was held.

  While the ***HOLD**** soft key is active during these prime procedures, the contents of the bottle continue to flow out of the bottle, through the valve block, and into the waste bottle.

After all the reagent lines are flushed, perform the next four steps to complete the instrument shutdown procedure.

7. When all the reagent primes are completed, switch the Pressure/Vent switch to "Vent."
8. Shut off the external gas supply at the gas tank regulator.
9. **If you are moving Synergy after the shutdown**, remove the waste line at the Waste port. Save the compression nut on the Waste line. Use a 5/8 in. wrench to screw the waste plug into the Waste port (Figure 2-15).

   If you are not moving Synergy after the shutdown, empty the waste bottle, but leave the waste tubing connected at the Waste port.
10. Press the power switch on the front of Synergy to turn it off.

Return to Operation after Shutdown

Use the following procedure to re-start Synergy after you have used the shutdown procedure. This procedure assumes that Synergy is still connected to the external pressurized gas tank.

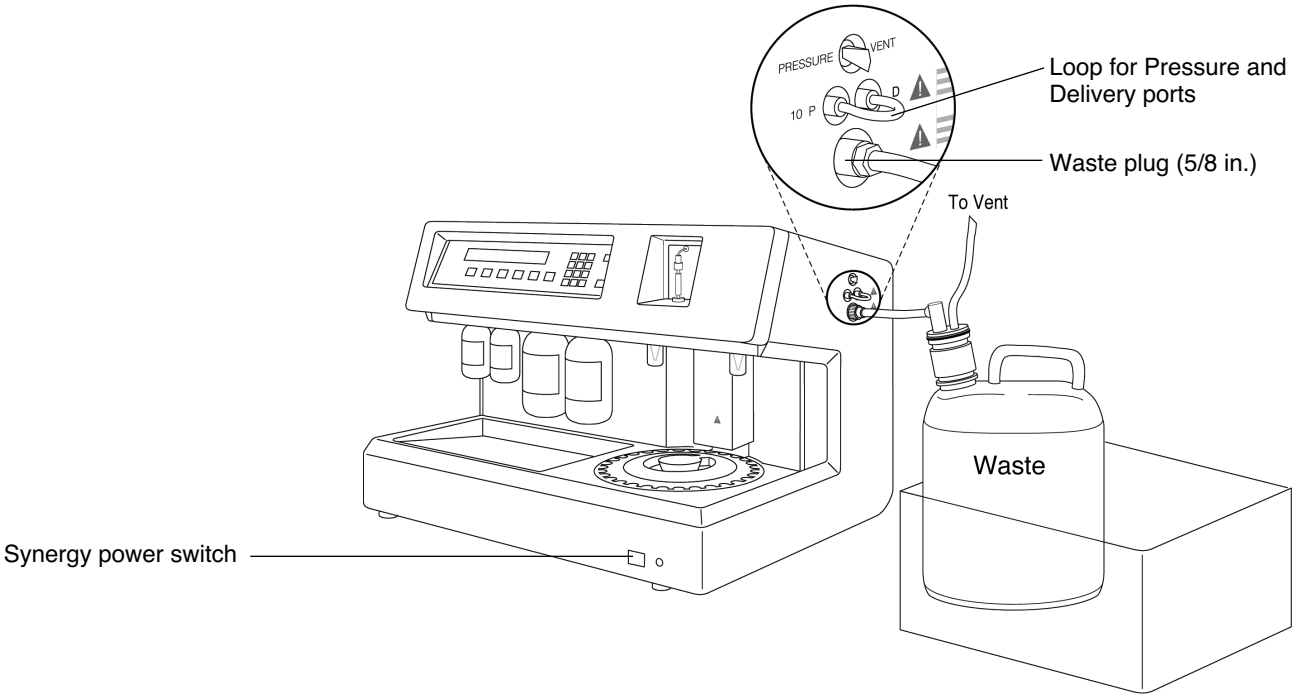


Figure 2-17. *Synergy after shutdown*

1. Turn on Synergy power switch.
2. If you have disconnected the waste bottle line, use a 5/8 in. wrench to remove the plug from the Waste port. Save the waste plug for future Synergy Shutdowns.

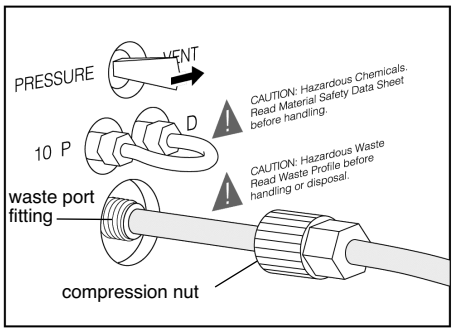


Figure 2-18. *Attaching the waste bottle line at the waste port fitting*

Place the plastic compression nut on the shorter tube from the waste bottle cap assembly (Figure 2-18). **Be sure the wide end of the ferrule goes on the tube first, so that the narrow end of the ferrule is closest to the waste port fitting.** Slip the tube into the Waste port and screw the plastic compression nut finger tight over the Waste port fitting.

Attach the longer tube from the cap assembly to the external ventilation system described in the Pre-Installation Manual and in the ***Laboratory Ventilation Requirements*** on page 1-8 of the Synergy Safety Guidelines. Check that the waste line runs up to the external ventilation, without any downward dips where waste can accumulate. If necessary, cut off excess line to allow proper flow.

3. Verify that the Pressure/Vent switch is in the Vent position.
4. Open the main supply valve on the gas tank and the regulator needle valve, if one exists. Turn on the external gas regulator. Adjust the external regulator pressure to 55 psi.
   a. Perform an external gas delivery leak test by turning the regulator knob 3-5 complete turns counterclockwise. As you turn the knob, the lower pressure gauge needle immediately drops 1-5 psi and then stabilizes. Note the reading on the lower gauge when it stops dropping. Wait five minutes.
   b. I**f the lower gauge reading has remained stable after 5 minutes**, there are no leaks in the external gas delivery system. Continue with step 5.
   c. **If the regulator needle moves down more than 1 psi in 5 minutes**, there is a leak in the external gas delivery line.

      Fill a large syringe with soapy water and squirt the solution around the connections between the regulator, Synergy, and the gas delivery line to locate leaks. Tighten any leaky connections.

      Repeat the external gas delivery leak test that starts at step a.

      If you cannot locate the source of the leak, do not continue this procedure. Call Applied Biosystems Technical Support for assistance.
5. Adjust the external gas tank regulator pressure to 65 psi.
6. With the Pressure/Vent switch in the Vent position, use a 1/4 in. wrench to remove the loop attached at the Pressure and Delivery ports. Attach the “D” (delivery) line from the DMF bottle cap to the Delivery port (Figure 2-17). Attach the “P” (pressure) line from the DMF bottle cap to the Pressure port.
7. Remove the 4 empty reagent bottles on the front of the instrument and replace them with full bottles of fresh reagents. (See ***Bottle Changing Procedure*** on page 4-9.) Replace any cracked or damaged bottle seals.
8. Run the Bottle Prime (***Priming Reagent Delivery Lines*** on page 4-11) for all 5 reagent bottles.

   If a pressure test failure occurs during a bottle prime, check the bottle seal and cap for that bottle.

9. **Use a marked flow test calibration tube** to perform Flow Test 3. (***Flow Tests 3, 4, and 5*** on page 4-22.) If Flow Test 3 passes, go to step 10.

   If you do not have a marked flow test calibration tube, perform Flow Tests 1 and 2, then perform Flow Test 3.

   If Flow Test 3 does not pass, perform Flow Test 1 (page 4-15).

   If Flow Test 1 does not pass, check the internal gas pressure (***How to monitor the internal gas pressure*** on page 4-23) and, if necessary, adjust the internal pressure to 5.0 ± 0.1 psi. Repeat Flow Test 1 and then perform Flow Tests 2 through 9. If Flow Test 1 continues to fail, call Applied Biosystems Technical Support.

10. If you have not performed Flow Test 4, do so now. (See ***Flow Tests 3, 4, and 5*** on page 4-22.) If Flow Test 4 does not pass, call Applied Biosystems Technical Support.

11. If both Flow Tests 3 and 4 pass, and you have not already done so, perform Flow Test 8 (page 4-27) and Flow Test 9 (page 4-30).

    When Flow Tests 8 and 9 pass, Synergy is ready to synthesize.

3 Post Synthesis Procedures

Contents

TFA Cleavage Procedure

WARNING **POTENTIAL EXPOSURE TO HAZARDOUS CHEMICALS.** ***Chemicals used in these procedures can be hazardous and can cause injury, illness, or death. Read the warnings prominently displayed on the bottle labels of all hazardous chemicals. Wear protective eye glasses, gloves and a laboratory coat when handling these chemicals. Always handle hazardous materials in a working fume hood.***

Cleavage is the process of chemically removing both the peptide from the resin and the side-chain protecting groups from the peptide. If you have no previous experience with cleaving a new peptide, we suggest you cleave on a small scale at first, with ***10 - 20 mg*** of peptide-resin and analyze the crude peptide with HPLC. You may cleave the entire contents of a peptide synthesis column, or 50 - 200 mg of peptide-resin.

In the instructions presented here, we describe glassware and reagent volumes for both small-scale and large-scale cleavages. Large-scale recommendations are printed in regular type with the corresponding small-scale recommendations in bold-face italics and in parentheses.

Two-stage cleavage procedure

This TFA cleavage procedure is described in two stages: the cleavage reaction, followed by crude peptide recovery. We describe two different recovery procedures: centrifugation and vacuum filtration. Choose either of these recovery methods, depending on the equipment you have access to and the applicable federal, state, and local laboratory regulations.

Post-cleavage recommendations

After cleavage, you may freeze-dry, or lyophilize, the peptide to remove water and volatile salts. If you don't have access to a lyophilizer, you may use a Speed-Vac™ centrifuge to remove liquid from the crude peptide in solution. To enhance removal of scavengers, you may dissolve the lyophilized peptide and re-lyophilize. Store the lyophilized peptide in a desiccator.

If your application requires further purification, the lyophilized peptide can be re-dissolved and then purified by reverse-phase HPLC.

Further reading

The information in this section is only an introduction to post-synthesis procedures. See page 3-8 for a list of references you can read for more information on post-cleavage analysis and purification of synthetic peptides.

Cleavage Reaction Procedure

WARNING	**CHEMICAL HAZARD.** ***Trifluoroacetic acid is an extremely dangerous and corrosive liquid. Always wear gloves, a lab coat, and eye protection when handling it.***

Note	***Thioanisole and EDT produce an unpleasant, offensive stench. Store and handle these reagents in a fume hood. To diminish odors, dispose of pipette tips that come in contact with these chemicals in a container of bleach.***

Required Reagents

TFA (Trifluoroacetic acid)	thioanisole
EDT (1, 2, ethanedithiol)	MTBE (methyl t-butyl ether)

Equipment and Labware

pliers or a decrimping tool (ABI P/N 400414)

polypropylene, graduated, 50-mL (***15-mL***) conical tubes with screw caps

orbital shaker with an attached tube rack

1. With one hand, firmly grasp the bottom of the peptide synthesis column (PSC) that contains the peptide-resin. With the other hand, use pliers or a decrimping tool to carefully pry off the column cap (Figure 3-1).

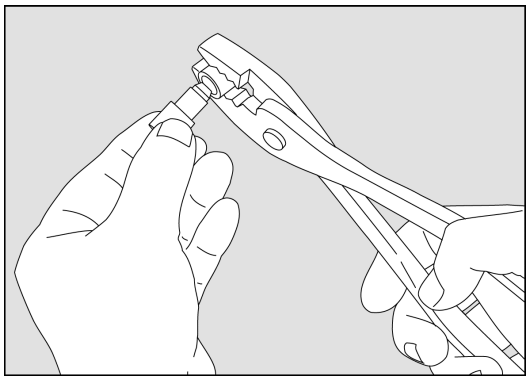


Figure 3-1. *Removing the PSC cap with pliers*

2. Place the entire contents of the peptide synthesis column (approximately 50 - 200 mg peptide-resin) in a 50 mL conical tube. (***For small scale, place***

10 - 20 mg peptide-resin in a 15 mL conical tube.) Tap the conical tube on a bench top to insure that all the resin falls to the bottom of the tube.

3. Working in a fume hood, first add 50 µL (5 µ***L***) thioanisole, and 50 µL (5 µ***L***) EDT. Lastly, add 900 µL (90 µ***L***) TFA.
4. Cap the tube and agitate it on an orbital shaker for one hour at room temperature. If an orbital shaker is not available, briefly shake the tube by hand once every 15 minutes for one hour.

Note *Increase the reaction time approximately one hour for each Arg(Pmc) in the peptide.*

5. Add 15 mL (***2 mL***) MTBE to the contents of the conical tube. **Tightly cap the conical tube** and vigorously vortex the contents to completely suspend the peptide in the MTBE solution. Vortexing should completely break up any sticky clumps of precipitated material from the sides of the tube.

Recover the crude peptide from the cleavage solution

Following step 5 of the preceding cleavage reaction procedure, you must separate the crude peptide from impurities and scavengers in the ether solution. Here we suggest two methods for recovering: centrifugation and filtration. In both procedures, impurities are first removed with multiple MTBE washes and then the peptide is dissolved in water or another solvent.

With the centrifugation procedure, you need no additional labware. In those laboratories where regulations forbid centrifugation of MTBE, the filtration procedure may be used as an alternative. To perform the filtration procedure, you need a vacuum system and filtration glassware.

Recovery by centrifugation

This procedure starts with the MTBE suspension of crude peptide from step 5 of the preceding cleavage reaction procedure.

WARNING **CHEMICAL AND FIRE HAZARD.** ***MTBE is volatile and highly flammable. Use only a spark-free centrifuge. Avoid prolonged centrifugation of MTBE. Perform this procedure in a working fume hood.***

Required Reagents

MTBE

glacial acetic, NH_4OH, or acetonitrile

distilled water

Equipment and Labware

disposable Pasteur-type pipettes and a rubber bulb

cotton balls (or glass wool)

microliter pipetters 200 µL and 1000 µL (***20 µL and 200 µL***)

clinical centrifuge (1500 g - 2000 g)

vortex mixer

1. Prepare a filter for step 6 of this procedure by pushing a small piece of cotton or glass wool into a disposable Pasteur-type pipette, as shown in Figure 3-2.

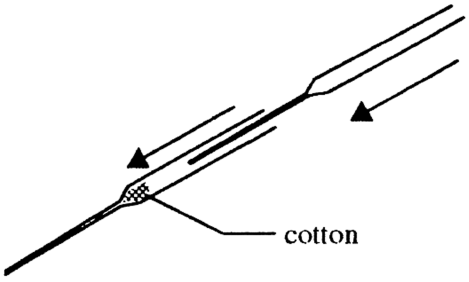


Figure 3-2. *Prepare a filter from a disposable pipette*

2. Centrifuge the tightly capped tube from the cleavage reaction for 2 - 3 minutes. The peptide and spent resin should form a pellet in the bottom of the tube. (See Figure 3-3.)

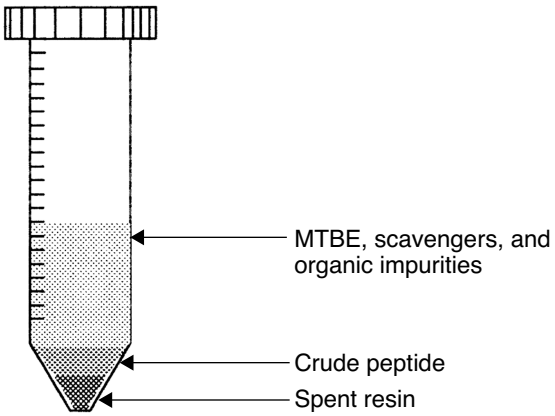


Figure 3-3. *Peptide-resin after MBTE wash and centrifugation*

3. Aspirate, or decant, and discard the MTBE.
4. Add 15 mL (***2 mL***) MTBE to the tube, tightly cap and vigorously vortex it to re-suspend the peptide. Repeat steps 2-4 at least three times.
5. After the final MTBE wash, decant or aspirate the MTBE and add 5 mL (***1 mL***) distilled water to the conical tube. Gently vortex the tube to dissolve the crude peptide.
   - If the peptide is basic, it will probably dissolve in H_2O. If it doesn't dissolve well in water, try adding a few drops of glacial acetic acid or acetonitrile. Do not let the concentration of glacial acetic exceed 15% (v/v), because high concentrations of acetic acid do not lyophilize.
   - If the peptide is acidic, dropwise add NH_4OH to solubilize.
   - If the peptide is hydrophobic, add acetonitrile to solubilize.
6. Separate the crude peptide solution from the spent resin by passing it through the filter (Figure 3-2) and into a second, 50-mL (***15 mL***), polypropylene tube.
7. Re-use the pipette and the filter to repeat Steps 5 and 6 two more times to give a total of 15 mL (***3 mL***) solution.
8. Place a lint-free tissue over the top of the tube and freeze the peptide solution by placing it in either liquid nitrogen or a container of isopropanol and dry ice. The peptide solution may now be lyophilized.

Recovery by vacuum filtration

This procedure starts with the MTBE suspension of crude peptide from Step 5 of the cleavage reaction procedure.

WARNING **CHEMICAL AND FIRE HAZARD*. MTBE is volatile and highly flammable. Perform this procedure in a working fume hood.***

Required Reagents

MTBE

distilled water

glacial acetic, NH_4OH, or acetonitrile

Equipment and Labware

a medium-porosity, fritted glass funnel (15 mL)

2 clean vacuum filtration flasks (125 mL)

Teflon™ or stainless steel spatula

vortex

1. Collect the peptide by filtering the contents of the graduated conical tube through a medium-porosity, fritted glass funnel mounted on a vacuum filtration flask.
2. Turn the vacuum off and add 10 mL MTBE to the contents of the funnel. Stir the contents with a spatula to resuspend the peptide and turn the vacuum on to filter again. Repeat this wash step three times.
3. Remove the filtration flask that now contains MTBE and impurities and discard the filtrate. Place the fritted funnel on a clean filter flask.
4. Add distilled water to the funnel to dissolve the peptide.
   - If the peptide is basic, it will probably dissolve in H_2O. If the peptide does not dissolve well in water, try adding a few drops of glacial acetic acid. Do not let the concentration of glacial acetic exceed 15% (v/v), because high concentrations of acetic acid do not lyophilize.
   - If the peptide is acidic, dropwise add NH_4OH to solubilize.
   - If the peptide is hydrophobic, add acetonitrile to solubilize.
5. When the peptide dissolves, turn on the vacuum to complete the filtration. Transfer the dissolved peptide from the filtration flask to a clean freeze-drying flask. Freeze the contents by placing the flask in either liquid nitrogen or a container of isopropanol and dry ice. The peptide solution may now be lyophilized.

Peptide Analysis and Purification: Recommended Reading

After cleavage, the synthesized peptide may be sufficiently free of impurities for many applications. The techniques most commonly used to analyze and purify synthetic peptides incorporate high performance liquid chromatography (HPLC), especially RPC (reverse-phase chromatography).

Each synthetic peptide has its own unique sequence and structural characteristics that contribute to its solubility behavior. These variables also influence the choice of HPLC techniques appropriate for analysis and purification. Discussing all the possible variables and compatible techniques would be an ambitious project, beyond the scope of this manual. Instead, we refer you to the following books and articles you may find useful.

Andrews, P.C., 1988. *Peptide Research,* 1:9.

CRC Press, 1984. *Handbook of HPLC for the Separation of Amino Acids, Peptides, and Proteins*

Guo, D., Mant, C.T., Taneja, A.K., Parker, J.M.R., and Hodges, R.S., 1986. *J. Chromatogr.,* 359:499.

Hoeger, C., Galyean, R., Boublik, J., McClintock, R., and Rivier, J., 1987. *BioChromatography*, 2:134.

Mant, Colin T., and Hodges, Robert S., eds. 1991. *High-Performance Liquid Chromatography of Peptides and Proteins: Separation, Analysis, and Conformation*, Boca Raton, Fla.: CRC Press.

Meek, J.L. and Rossetti, Z.L., 1981. *J. Chromatogr*., 211:15.

Rivier, J. and McClintock, R., 1983. *J. Chromatogr*., 268:112-119.

Tsarbopolous, A., 1989. Peptide Research, 2(4): 258-266.

4 Maintenance and Self Tests

Contents

Maintenance of the Synergy Peptide Synthesizer

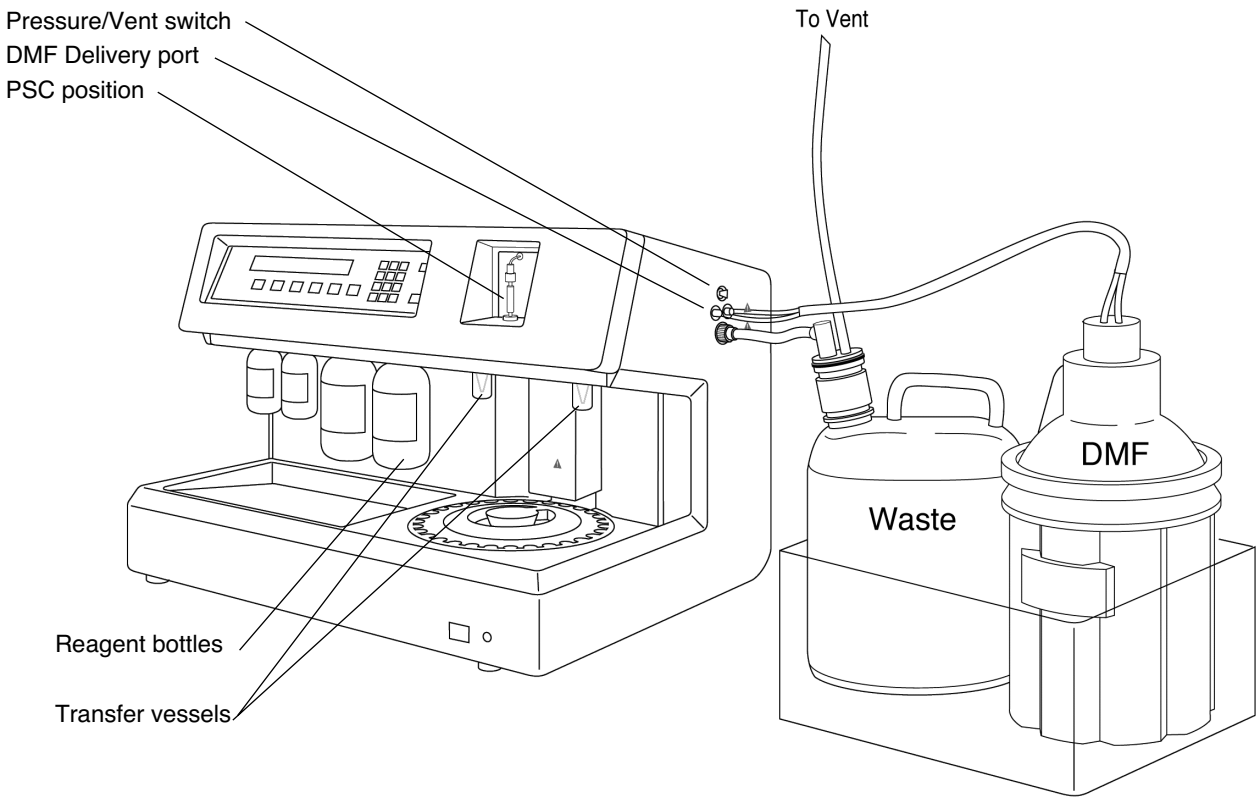


Figure 4-1. *Synergy after installation (printer not shown)*

The Synergy Peptide Synthesizer requires little maintenance. This chapter contains descriptions of some minor adjustments you can make and the Self Tests. Most of the time, you will probably use only two Self Tests: the Bottle Self Test (page 4-9), to change reagent bottles; and Flow Test 8: Calibrate HBTU (page 4-27), to calibrate HBTU delivery after changing the delivery line filter for that reagent. Procedures in Chapter 7, ***Troubleshooting***, may recommend performing other Self Tests under certain conditions.

All operations described in this chapter can be performed without removing the instrument's exterior panels. If a Self Test repeatedly fails, there may be a hardware malfunction inside the synthesizer that can best be repaired by a trained Applied Biosystems Service representative. If a Self Test repeatedly fails, call Applied Biosystems Technical Support for assistance.

Adjusting the Conductivity Trace Printout

In the Chart Recorder Menu you may make three different modifications to the appearance of the conductivity trace:

- Change the scales of the two overlapping trace lines.
- Change the chart speed.
- Make the date, time, and cycle number appear before each cycle on the trace.

Note *Although you may make these modifications while the printer is printing a trace, the changes will not show up on the trace until you re-start data printing.*

You can make these modifications at any time, however, the modifications do not go into effect until you begin data printing. If Synergy is already printing data when you make changes, either cancel printing (see ***How to replace or re-align printer paper during synthesis*** on page 2-23) or wait till the end of the current data printout to restart printing and see how the printout has been modified.

How to change the scales on the conductivity trace printout

1. In the Main Menu, press the ***setup & report*** soft key to go to the Setup & Report Menu.

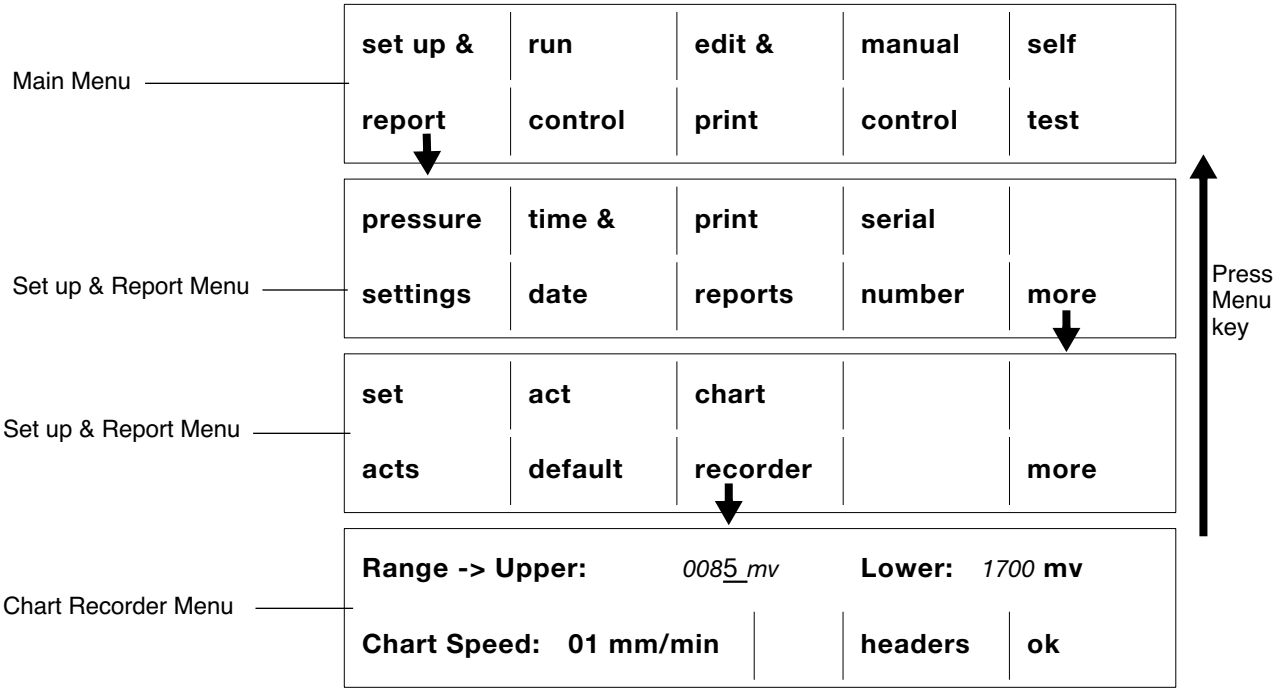


Figure 4-2. *How to go to the Chart Recorder Menu*

2. In the Setup & Report Menu, press the ***more*** soft key until the ***chart recorder*** soft key becomes available (Figure 4-2). Press the ***chart recorder*** soft key to go to the Chart Recorder Menu.

   The top line in the Chart Recorder Menu has entry fields you can use to modify the scales of the two conductivity trace lines. The Upper mv (millivolt) values corresponds to the trace line for Trace Features 1 through 4 and Trace Feature 7 on the conductivity trace (see Figure 2-7 on page 2-17 for examples of all the Trace Features). The Lower mv value corresponds to the trace line for Trace Features 5 and 6.

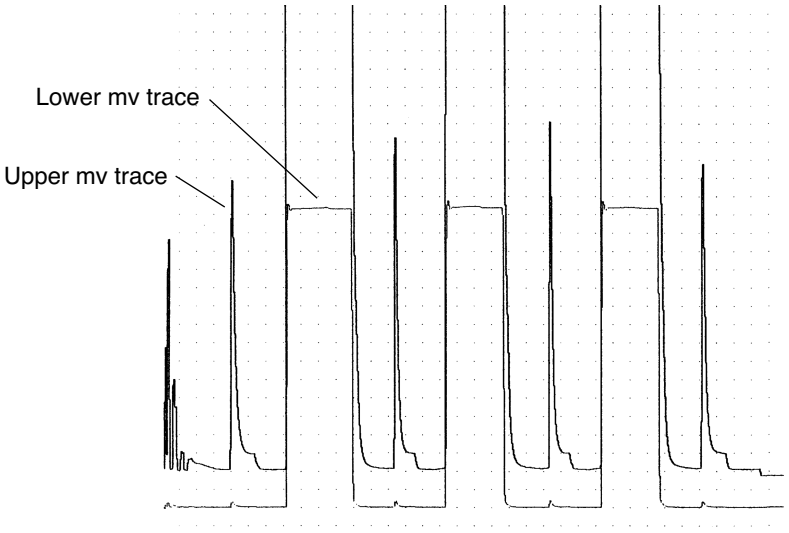


Figure 4-3. *The Upper and Lower conductivity trace lines*

3. Use the arrow key to move the cursor to either the Upper or Lower mv value. Use the alphanumeric keys to increase or decrease the value.

   When you increase the value, you increase the range of millivolts represented by the trace. So, if the conductivity trace runs off the page, increase the range to confine the trace within the page limits.

   When you decrease the value, you decrease the range of millivolts represented by the trace. Decrease the value when the peaks on the conductivity trace are too short.

4. Press the Menu key to move out of the Chart Recorder Menu.

How to change the chart speed

In addition to the conductivity trace lines, every printout has evenly spaced background dots. The space between each vertical row of dots represents 5 minutes of data.

The chart speed has two settings: 1 mm/min and 2 mm/min. A trace printed at a chart speed of 1 mm/min is half the width and uses half the amount of paper than the same

trace printed at 2 mm/min. The height of the trace is not effected by changing the chart speed, nor does the time it takes to print data during a synthesis change when the chart speed changes.

1. In the Chart Recorder Menu (Figure 4-2 on page 4-4), press the (→) arrow key to move the cursor from the top line to the bottom line of the LCD. Place the cursor in the field after the words "Chart Speed."
2. Use the alpha numeric keys to enter either 1 (one) or 2 mm/min.
3. Press the ***OK*** soft key to move out of the Chart Recorder Menu.

How to make the date, time, and cycle number appear on the printout

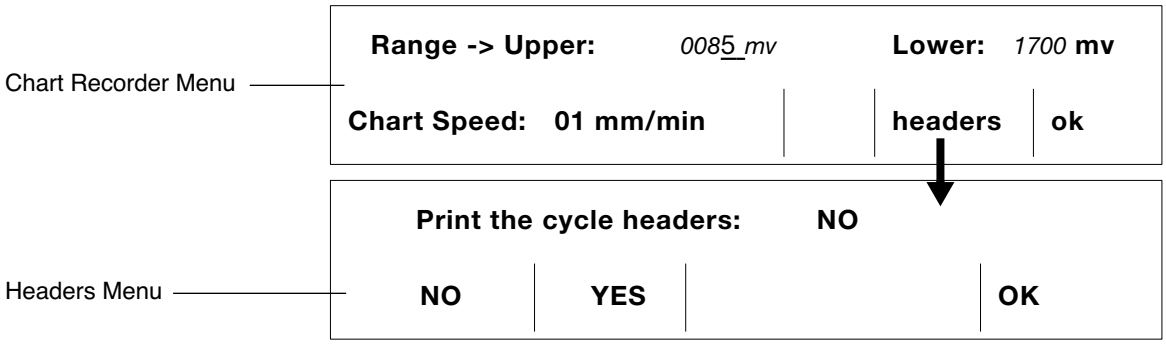


Figure 4-4. *How to go to the Headers Menu.*

When you choose headers, Synergy prints the date and time each cycle started with the cycle number. If you use a pre-loaded PSC (one that contains an amino acid) cycle one corresponds to the deprotection of the C-terminal amino acid and coupling of the second amino acid from the C-terminal.

1. Go to the Chart Recorder Menu (Figure 4-2 on page 4-4).
2. Press the ***headers*** soft key in the Chart Recorder Menu to go to the Headers Menu.
3. Press the ***Yes*** soft key if you want headers on the printout; press the ***No*** soft key if you don't want headers on the printout.
4. Press the ***OK*** soft key to move out of the Headers Menu. Press the ***OK*** soft key to move out of the Chart Recorder Menu.

Changing Synergy Fuses

Two 2 A/250 V SB (Slo Blo® or equivalent) fuses protect Synergy's interior electrical circuitry against power surges and excessive power consumption. If the fuses blow soon after they have been replaced, there could be a short circuit inside the instrument. Call Applied Biosystems Technical Support if this happens.

The fuse holder is part of the AC power input module that includes the 3-prong power cord receptacle.

How to change fuses

1. Turn off the power on the front of Synergy and unplug the power cord.
2. To remove the fuse holder, use a screwdriver blade to push the flexible plastic, snap-tab closure to the left. When the tab is pushed far enough to the left, the fuse holder pops out.

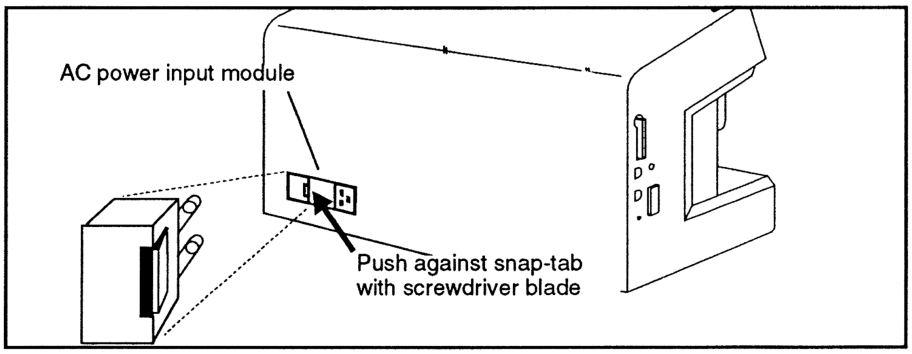


Figure 4-5. *Locate the fuse holder on the rear of the Synergy Peptide Synthesizer 3.*

3. Pull out and remove the two blown fuses.
4. Insert two, 2A/250 V (Slo Blo® or equivalent) fuses into the two round openings in the fuse holder.
5. Push the fuse holder back in place into the AC power input module. The fuse holder snaps firmly in place so that the back of the fuse holder is flush with the back instrument panel.
6. Plug in the power cord and press the power button on the front of Synergy to turn the power on.

Self Test Menus

When you run a self test, a set of events automatically occurs to test a specific part of Synergy's hardware. As the test progresses, the LCD may display instructions that you must follow to complete the test. When the test is finished, the LCD announces whether or not the test passed.

The following are the seven types of self tests:

bottle: consists of two categories, CHANGE and PRIME, for changing reagent bottles and priming reagent delivery lines

leak: checks for leaks in the pressure system

flow: consists of nine flow tests used to check reagent deliveries

cycle: runs a synthesis of the test peptide LAGV

keyboard: checks that all the keys for the LCD are working

wheel: tests the pressurized autosampler jaws and the amino acid column wheel

circuit: tests the electrical circuitry to the valves and the ROM card

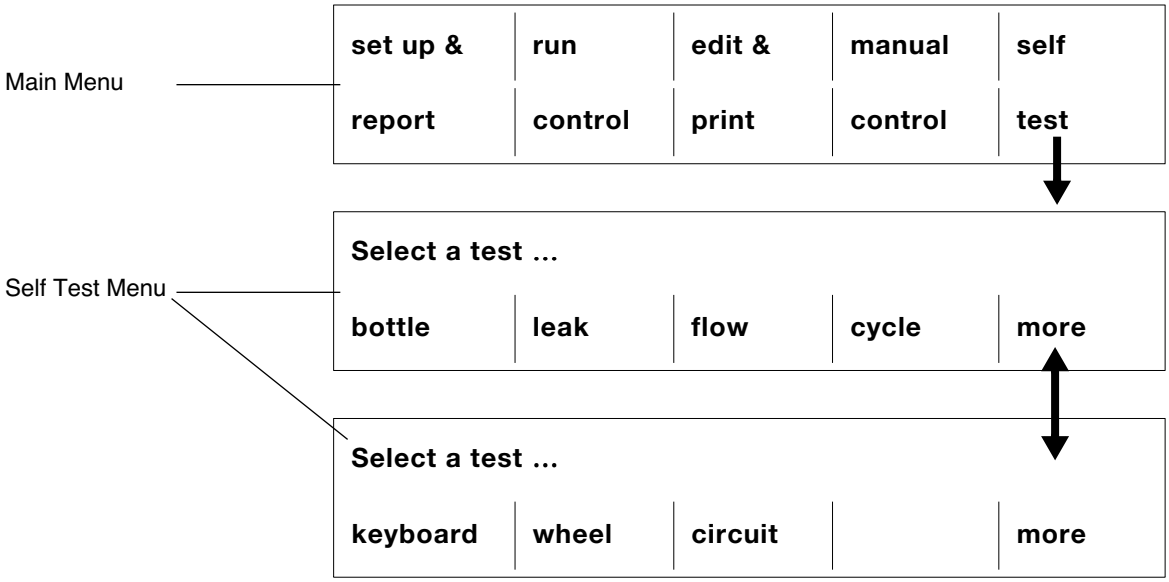


Figure 4-6. *The Self Test Menu*

- Press the ***self test*** soft key in the Main menu to go to the Self Test Menus.
- Press the ***more*** soft key to toggle between the two Self Test Menus.
- Press the Main key to return to the Main Menu.

Bottle Self Test

Change and prime the bottle(s)		
CHANGE	**prime**	

- Press the ***CHANGE*** soft key before you replace any empty reagent bottles.
- Press the ***prime*** soft key to flush reagent delivery lines with reagent.

Bottle Changing Procedure

Use this bottle change procedure to change one or more of the following four reagent bottles: HBTU, DIEA, THF, or DMF.

WARNING **CHEMICAL HAZARD** ***Reagent bottles are pressurized. With the exception of piperidine, always switch the Pressure Vent switch to "Vent" before unscrewing a reagent bottle on the Synergy Peptide Synthesizer.***

Piperidine is the only reagent that is on its own pressure system, separate from the other bottles. You may open or replace the piperidine bottle without depressurizing the pressure system for the other reagents. When you open the piperidine bottle, you may hear a small hiss as pressure is released. **Do not open the piperidine bottle when Synergy is running module d**.

1. To begin the bottle changing procedure, press the ***bottle*** soft key in the Self Test Menu (Figure 4-6). Then press the ***CHANGE*** soft key.

Switch the Pressure/Vent to Vent.	
	OK

2. Before removing any bottles, set the Pressure/Vent switch to Vent. Press the ***OK*** soft key. During the 30-second interval that follows, the internal pressure drops to zero.

Replace empty reagent bottles with full	
reagent bottles.	**OK**

3. Remove the empty reagent bottles and the bottle seal for each bottle on the front of Synergy. You may have to use tweezers to dislodge the bottle seal from inside the ratchet cap. The DMF bottle seal is built into the DMF bottle cap assembly.

   Always use new bottle seals. Bottle seals should never be used more than once. Note that there are two sizes of bottle seals.

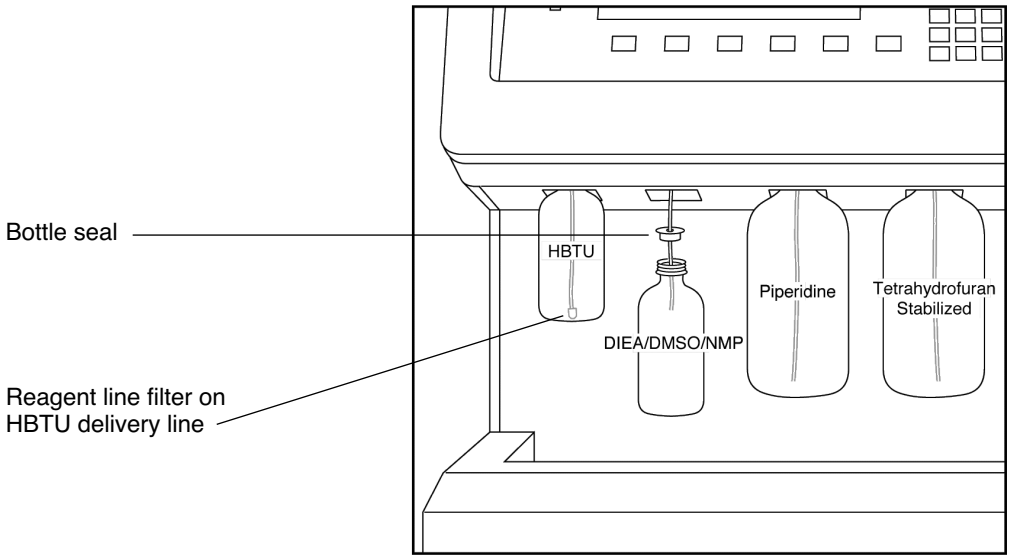


Figure 4-7. *Reagent bottle positions on the front of the Synergy Peptide Synthesizer.*

4. Open the full reagent bottle and check the rim of the bottle for chips. With the exception of the DMF bottle, place a bottle seal on the rim.

Note	***You must combine the contents of two reagent bottles to prepare the HBTU reagent. Instructions for preparing the HBTU reagent are found on page 2-7.***

If you are replacing one of the four bottles on the front of the instrument, hold the bottle straight up, with its reagent delivery line inside, and turn it carefully into the threads of the ratchet cap on Synergy (Figure 4-7). The bottle should fit firmly in place.

Caution	***Do not overtighten the reagent bottles! If you turn the bottle past the first point of resistance, you may crack the bottle seal or damage the ratchet cap assembly.***

When all the reagent bottles are in place, from left to right, facing the front of the synthesizer, the labeled reagent bottles should be HBTU [2-(1H-benzotriazol-l-yl)-1,1,3,3 -tetramethyluronium hexafluorophosphate] in

position A, DIEA/DMSO/NMP (0.4M N,N-diisopropylethylamine/dimethyl sulfoxide/N-methylpyrrolidone) in position B, Piperidine in position 1, and THF (Tetrahydrofuran) in position 2, DIEA, Piperidine, and THF (Figure 4-7). The DMF (N, N-dimethylformamide) bottle sits on the right side of Synergy.

5. When all the reagent bottles are in place, press the ***OK*** soft key.

6. Switch the Pressure/Vent switch to "Pressure" and press the ***OK*** soft key. A three-minute pressure test follows.

7. When this display appears, all the bottle connections are tight. Press the ***OK*** soft key.

   If this test fails, one or more of the reagent bottles have not been properly attached to Synergy. Read ***What to do after a pressure test failure*** on page 7-12.

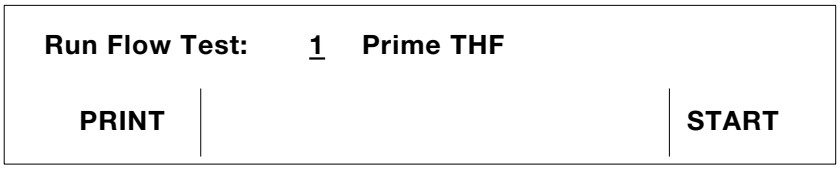
Priming Reagent Delivery Lines

There are 5 priming flow tests, one for each of the reagent bottles. During each priming routine, a pressure test checks for leaks.

Table 4-1. Priming Flow Tests

Test Number	Bottle Primed
1	THF
2	DMF
3	HBTU
4	DIEA
5	Piperidine

1. Set the Pressure/Vent switch to Pressure before beginning any prime.
2. To begin the priming procedure, press the ***bottle*** soft key in the Self Test Menu (Figure 4-6). Then press the ***prime*** soft key.



3. Press the *Next* key or the *Previous* key until the appropriate priming flow test appears on the LCD.

   As an alternative, you may use the alphanumeric keys to enter the number of the priming flow test you wish to run (Table 4-1).

4. Press the ***START*** soft key.

5. When this display appears, no leaks have been detected and the priming procedure is completed. Press the ***OK*** soft key.

6. If a pressure leak is detected during the bottle prime procedure, see ***What to do after a pressure test failure*** on page 7-12.

Leak Self Test

Use the Leak Self Test to check the entire internal pressure system for leaks. You need the calibration test fixtures to perform the Leak Self Test.

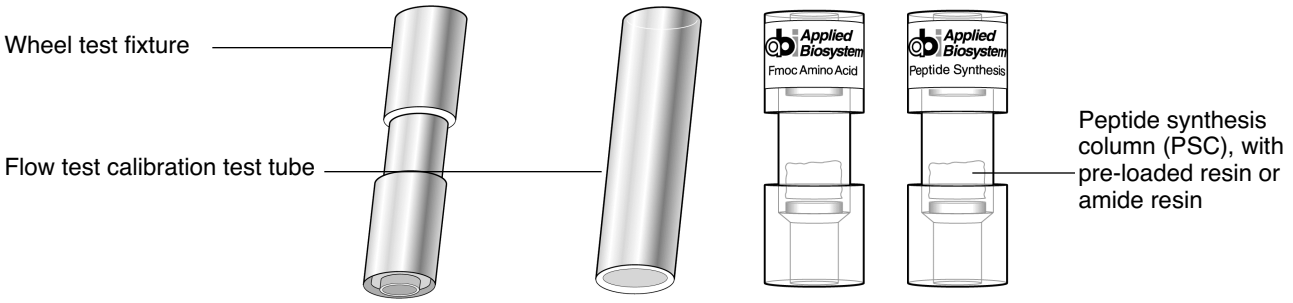
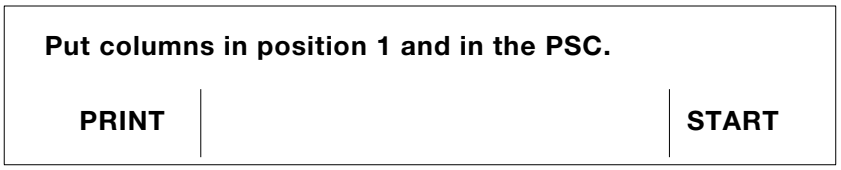


Figure 4-8. *Calibration Test Fixtures, Amino Acid, and Peptide Synthesis Columns*

How to perform a Leak Self Test

1. To begin the Leak Self Test, press the ***leak*** soft key in the Self Test Menu (Figure 4-6).



2. Place a wheel test fixture in position one (1) on the amino acid column wheel (Figure 4-9).

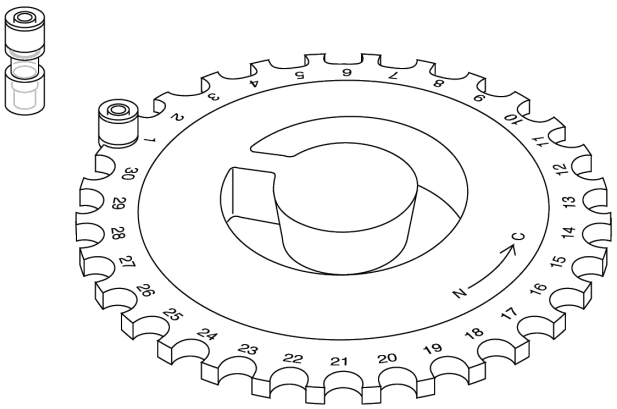


Figure 4-9. *Placing a wheel test fixture in position one*

3. Place the wheel on top of the hub on the right side of Synergy. Slowly rotate the wheel until the protruding pin on the bottom of the wheel slips into the corresponding groove in the hub.

4. Place a flow test calibration tube in the peptide synthesis position (Figure 4-10).

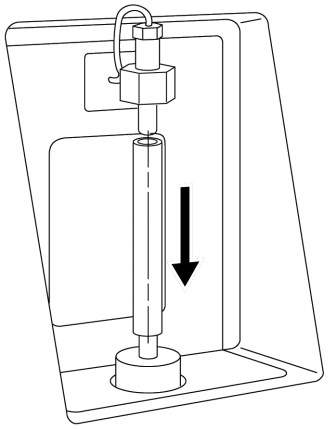


Figure 4-10. *Placing a flow test calibration tube in the peptide synthesis position.*

Twist and push the luer fittings on at both ends of the flow test calibration tube. **Do not unscrew the black plastic bushing attached to the top liner fitting**.

Caution	***Leaks at the peptide synthesis column position can damage the conductivity cell. To assure a leak-proof connection, push the luer fittings onto both column ends with a slight twisting motion.***

5. Press the ***START*** soft key.

WARNING	**PERSONAL INJURY HAZARD.** ***The autosampler jaws are operated by a pneumatic valve under high pressure. Keep your fingers away from the jaws during the Leak Self Test and any other time the jaws are in operation.***

During the Leak Self Test, eight different pressure tests are performed. If a pressure leak is detected, the LCD displays the message “Pressure test failed:”. The second line of the LCD reports the pressure readings that were taken when the pressure test failed. Refer to ***What to do after a pressure test failure*** on page 7-12.

Test Passed.	
	OK

If no pressure leaks are detected, the LCD displays the message "Test passed." Press the ***OK*** soft key to complete the Leak Test.

Flow Tests

Use flow tests to test and calibrate the flow of chemicals through the instrument. Perform Flow Test 8 every time you change the delivery line filter on the line to the HBTU bottle. Procedures in Chapter 7, ***Troubleshooting***, direct you to use certain flow test for specific conditions. To perform these Flow Tests 1, 8, and 9, you must have access to a scale that is accurate to ± 1 mg.

If you want a list of the steps in any flow test, turn on the printer and press the ***PRINT*** soft key at the beginning of the flow test.

Table 4-2. Flow Tests

Test Number	Test Name	Description
1	DMF → L/R/PSC	DMF to each transfer vessel and to the peptide synthesis column
2	CALIBRATE TUBE	DMF to flow test calibration tube
3	DMF → PSC	DMF to flow test calibration tube
4	THF → PSC	THF to flow test calibration tube
5	PIP → PSC	Piperidine to flow test calibration tube
6	Pump/ AAC	DMF to each transfer vessel and to the amino acid column
7	Pump/Cell	DMF and HBTU conductivity test
8	CALIBRATE HBTU	Calibrate HBTU delivery to left transfer vessel
9	CALIBRATE DIEA	Calibrate DIEA delivery to left transfer vessel

If any of these flow tests repeatedly fail, call Applied Biosystems Technical Support for assistance.

Flow Test 1: DMF Delivery to Vessels and Peptide Synthesis Column

This flow test checks three deliveries of DMF: first, through the top valve block; second, through the bottom valve block; and third, through the synthesis column.

During this flow test, you first tare the left transfer vessel and then, when prompted, weigh the vessel after each of three DMF deliveries and enter the values. If the values you enter do not meet specifications, the flow test fails and you must repeat the test.

IMPORTANT ***Always tare the left transfer vessel at the beginning of Flow Test 1, especially if you repeat the test.***

1. To begin Flow Test 1, press the ***flow*** soft key in the Self Test Menu (Figure 4-6).

Run Flow Test: 1 DMF → L/R/PSC	
PRINT	START

2. Verify that a flow test calibration tube is in place in the peptide synthesis position. Press the ***START*** soft key.

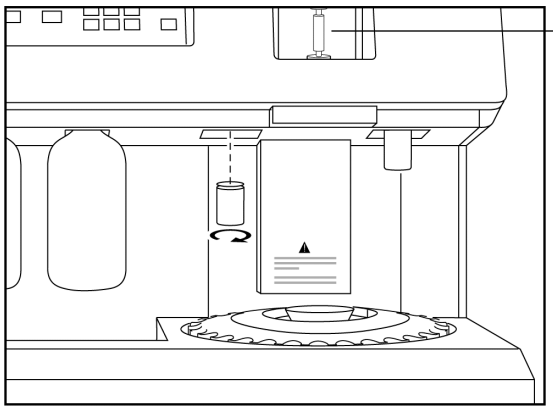


Figure 4-11. *Remove the left transfer vessel*

Remove the left vessel and put on scale.	
Zero scale then replace the vessel	OK

3. Remove the left transfer vessel (Figure 4-11) and place it on a scale that is accurate to ± 1 mg. Set the scale to zero. Replace the vessel on Synergy and press the ***OK*** soft key.

Remove the left vessel and weigh. _mg	
	OK

Note *This message appears 3 times during Flow Test 1. When it appears, the left vessel contains DMF.*

4. Carefully remove and weigh the left vessel and enter the weight on the LCD. Do not discard the DMF.

   Press the ***OK*** soft key.

5. Replace the left vessel and press the ***OK*** soft key.
6. When prompted, repeat steps 4 and 5 two more times.

   The values for the first two weights should be similar—460 - 740 mg—because they both measure DMF deliveries through the valve blocks. The third value results from DMF delivery through the PSC (peptide synthesis column) and should be 500 - 600 mg.

7. When this display appears the values are within specifications. Press the ***OK*** soft key to complete the flow test.

   If any of the DMF delivery weights is out of range, the LCD displays the message:

   When this display appears, press the ***OK*** soft key. The flow test continues to ask for weights until all three deliveries have been weighed. At the end of the test the LCD displays the message ***Test failed***.

If Flow Test 1 fails, repeat the test one more time.

- Always re-tare the left transfer vessel when prompted.
- Weigh the DMF **immediately** after each delivery to minimize weight loss due to evaporation or spillage.
- During the repeat, write down the value of the **third** DMF delivery weight. If the repeat of Flow Test 1 fails, adjust the internal gas pressure to calibrate the **third** DMF delivery.

If the flow test continues to fail:

- Check that the DMF reagent bottle is at least half full. Occasionally, a low volume of DMF in the reagent bottle may cause Flow Test 1 failure.
- Perform a Leak Test (page 4-13) to check for internal system leaks.
- Adjust the internal pressure to calibrate the **third** DMF delivery. Although the value of the third DMF delivery weight is acceptable in the range of 550 ± 30 mg, it is best to set the delivery as close to 550 as possible, typically 550 ± 10 mg. The acceptable internal gas pressure range is 3.5-5.5 psi.

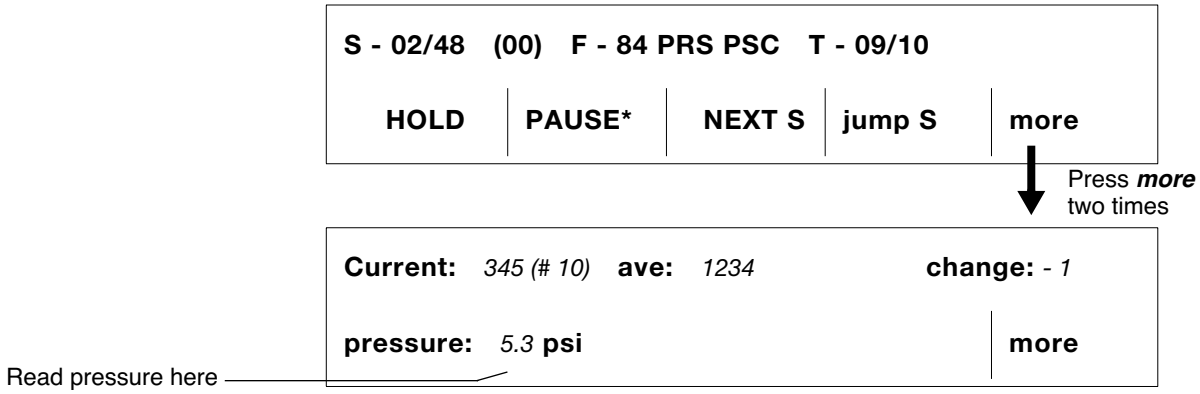
  If the third DMF delivery weight was less than 520 mg, increase the internal gas pressure.

  If the third DMF delivery weight was greater than 580 mg, decrease the internal pressure.

Adjusting the internal gas pressure to calibrate DMF delivery

1. Press the ***START*** soft key to begin Flow Test 1. ***Immediately*** press the ***PAUSE*** soft key to temporarily interrupt the flow test.

   When you press the ***PAUSE*** soft key, the current step number should be either ***02*** or ***03***. An asterisk appears (***PAUSE****) to indicate the flow test has been temporarily interrupted.



2. Press the ***more*** soft key **twice**, until the Run Monitor displays the current pressure reading on the bottom line of the LCD.

   If the pressure is too low, use a flat edged screwdriver to adjust the internal pressure to 3.5-5.5 psi. Insert the screwdriver blade into the small hole located behind the reagent bottles (Figure 4-12). A quarter turn clockwise raises the pressure reading by about 0.2 psi. Turn the screw clockwise, **in one direction only**, and wait 30 seconds for the pressure reading to stabilize.

IMPORTANT *Turn the internal gas adjustment screw in one direction only and then wait 30 seconds for the pressure to stabilize.*

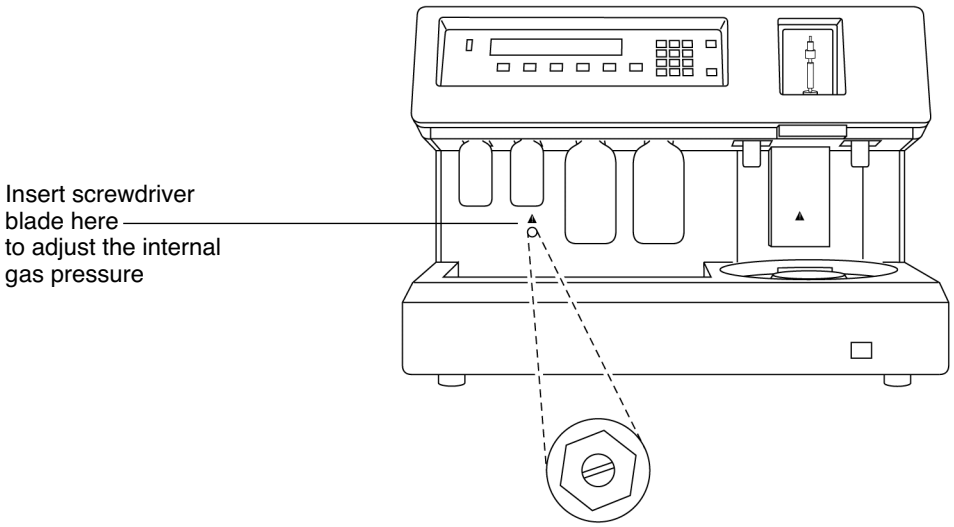


Figure 4-12. *Adjusting the internal gas pressure*

If the pressure is too high:

a. Note the current internal gas pressure reading that appears after the word ***pressure*** on the LCD.

b. Set the Pressure/Vent switch to "Vent" to vent the excess pressure.

c. Turn the pressure adjustment screw one quarter turn counterclockwise to lower the pressure by 0.2 psi.

d. Set the Pressure/Vent switch to "Pressure" and wait 30 seconds.

3. After you have adjusted the internal gas pressure, press the ***more*** soft key to return to the Run Monitor that displays the ***PAUSE**** soft key.
4. Press the ***PAUSE**** soft key to continue Flow Test 1.

   As Flow Test 1 continues, repeat the complete procedure to verify that all deliveries are within range. If all delivery weights are within range, Flow Test 1 passes.

   If any delivery weights are not in range, repeat the internal gas pressure adjustment procedure.

Flow Test 2: Calibrate Tube

During this test, two deliveries of DMF go into a flow test calibration tube placed in the peptide synthesis column position. You need an indelible ink marker to mark the level of DMF in the column after each flow. The marked flow test calibration tube is used to perform Flow Tests 3, 4, and 5.

1. Place a flow test calibration tube in the PSC position.
2. To begin Flow Test 2, press the ***flow*** soft key in the Self Test Menu (Figure 4-

6). Then press the *Next* key or press the numeric key "2" to make ***Run Flow Test: 2*** appear on the LCD.

Run Flow Test: 2 CALIBRATE TUBE		
PRINT		START

3. Press the ***START*** soft key. In the next two minutes, Synergy performs a brief pressure test and delivers DMF to the flow test calibration tube.

Mark the tube.	
	OK

4. Make a thin, horizontal mark on the flow test calibration tube to indicate the level of the DMF in the column (Figure 4-13). The mark should be even with the bottom of the meniscus. Press the ***OK*** soft key.

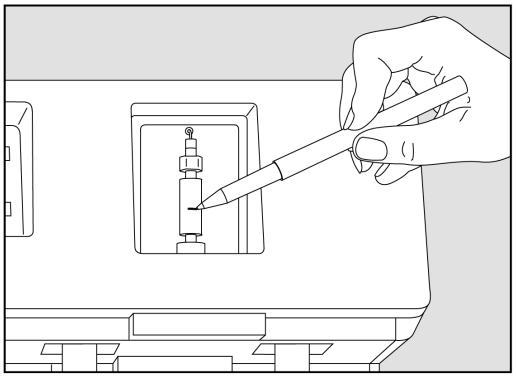


Figure 4-13. *Marking the flow test calibration tube*

5. After the second DMF delivery, the LCD directs you to make a second mark on the flow test calibration tube. The second mark should be higher than the first mark and even with the bottom of the meniscus.

IMPORTANT ***Save this marked flow test calibration tube; it will be used again in other flow tests.***

6. When this display appears, press the ***OK*** soft key to complete the flow test.

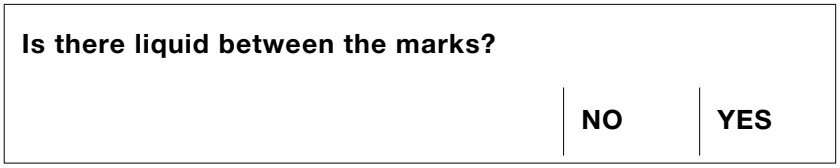
   If a pressure test failure occurs during this flow test, see ***What to do after a pressure test failure*** on page 7-12.

Flow Tests 3, 4, and 5

- **DMF → PSC**
- **THF → PSC**
- **PIP → PSC**

Each of these three flow tests delivers reagent to the flow test calibration tube that you marked in Flow Test 2. During each flow test, observe the meniscus of the reagent delivered to the flow test calibration tube. The meniscus should fall on one of, or between, the two marks on the tube.

1. To begin one of these flow tests, press the ***flow*** soft key in the Self Test Menu (Figure 4-6). Then press the *Next* key, the *Previous* key, or the numeric key that corresponds to the flow test number.
2. When the appropriate flow test appears on the LCD, press the ***START*** soft key to begin the flow test.



3. If the reagent meniscus is on one of, or between, the two calibration marks on the flow test calibration tube, press the ***YES*** soft key.

   If the reagent meniscus does not fall on one of, or between, these two marks, press the ***NO*** soft key.

   After 2 more minutes, the LCD reports if the test was successful.

4. Press the ***OK*** soft key to complete the flow test.

If any of these three flow tests fails:

- Check that each of the three reagent bottles contains fluid and that the ends of the reagent lines in the bottles extend to the bottom of the bottles.

- Prime the reagent line for the reagent(s) that fail. (See ***Priming Reagent Delivery Lines*** on page 4-11.)
- Check that the flow test calibration tube fits tightly in the luer fittings and re-run Flow Test 2.
- If a pressure test failure occurs during one of these flow tests, see ***What to do after a pressure test failure*** on page 7-12.

If Flow Test 3: DMF fails:

- Perform Flow Test 1, then repeat Flow Test 3. If Flow Test 3 continues to fail, call Applied Biosystems Technical Support.

If Flow Test 4: THF fails:

- If the THF level is near the marks in the flow test calibration tube during Flow Test 4, THF delivery is probably sufficient. At the end of a synthesis, THF delivery to the PSC washes the peptide-resin with volatile solvent. If the contents of the PSC after a complete synthesis are dry, there is no need to increase THF delivery.

  If THF delivery is inadequate and Flow Test 4 continues to fail, call Applied Biosystems Technical Support.

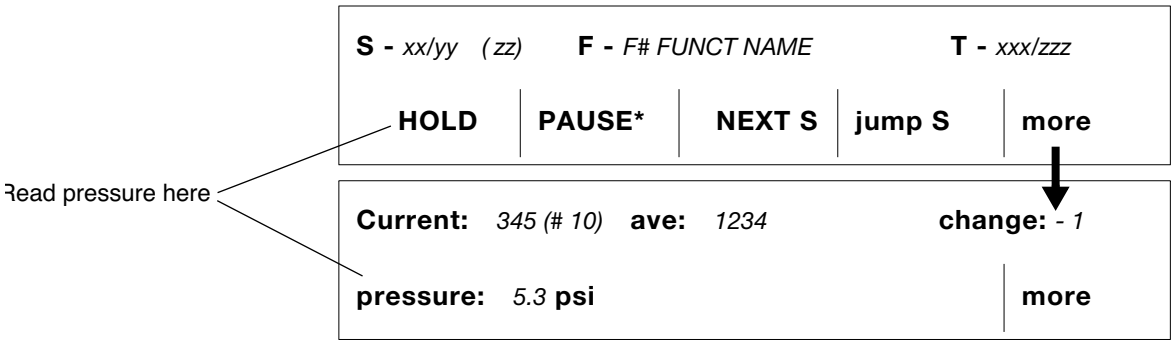
If Flow Test 5: PIP fails:

- If piperidine delivery is below the lower calibration mark, check the bottle seal on the reagent bottle and prime the PIP reagent line (page 4-11).
- If piperidine delivery is above the higher calibration mark, check the internal gas pressure reading. The current internal gas pressure should be 3.5-5.5 psi.

How to monitor the internal gas pressure

The internal gas pressure is set to 3.5-5.5 psi during Synergy installation. This pressure is fairly stable and requires little adjustment during routine operation.

During a synthesis, you can monitor the internal gas pressure in the Run Monitor.



- Press the ***more*** soft key until the Run Monitor displays the pressure reading on the bottom line. The top line displays conductivity readings.

When Synergy is not running a synthesis, use the following procedure to monitor the internal gas pressure.

In the Main Menu, press the ***set up & report*** soft key (Figure 4-14).

In the Set up & Report Menu, press the ***pressure settings*** soft key.

The minimum and maximum pressure settings appear on the top line of the Pressure Settings display. The current pressure appears on the bottom line of the LCD.

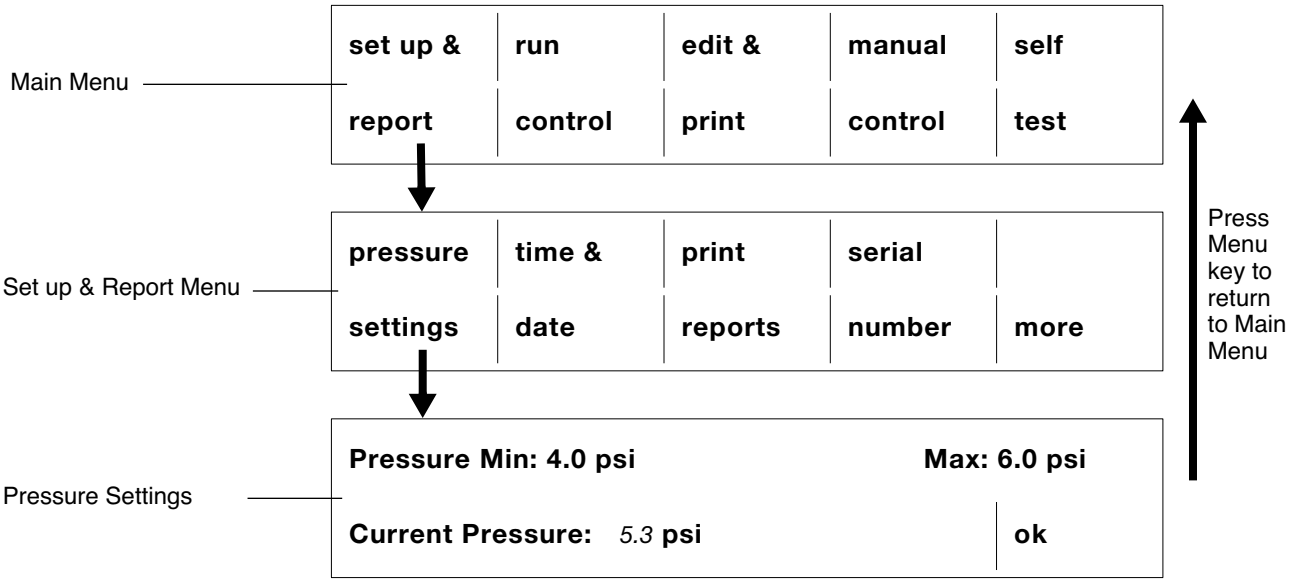


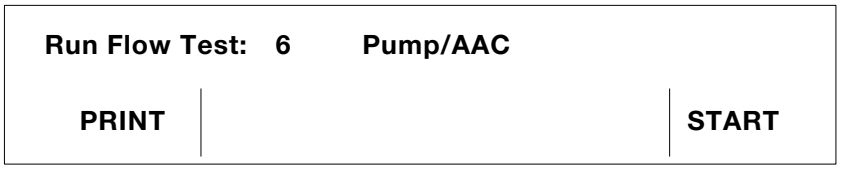
Figure 4-14. *How to monitor the current pressure*

To adjust the internal gas pressure, see Step 2. of the procedure ***Adjusting the internal gas pressure to calibrate DMF delivery*** on page 4-18.

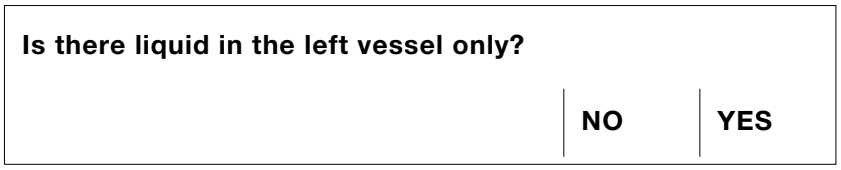
Flow Test 6: Pump/AAC

This test first checks the flow of reagent from the left transfer vessel, through the column on the amino acid column wheel, to the right transfer vessel. Then it checks the flow from the right transfer vessel back to the left transfer vessel. The "pump" in the name of this flow test refers to the configuration of transfer vessels that circulates liquids during synthesis.

1. Place a wheel test fixture in position 1 (one) on the amino acid column wheel.
2. To begin Flow Test 6, press the ***flow*** soft key in the Self Test Menu (Figure 4-6). Then press the *Next* key or press the numeric key "6" to make ***Run Flow Test: 6*** appear on the LCD.



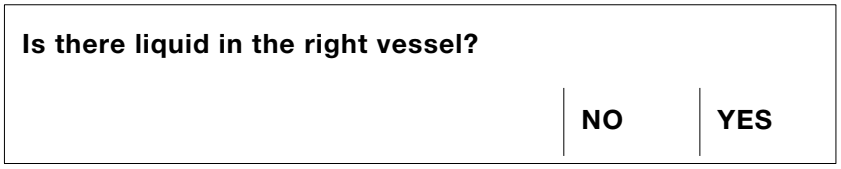
3. Press the ***START*** soft key to begin the flow test. A brief pressure test and a delivery of DMF to the left vessel follows.



4. Look at both transfer vessels. You should see liquid in the left transfer vessel. If you see liquid in only the left vessel, press the ***Yes*** soft key.

   If there is no liquid in the left transfer vessel, or if there is liquid in the right transfer vessel, press the ***No*** soft key.

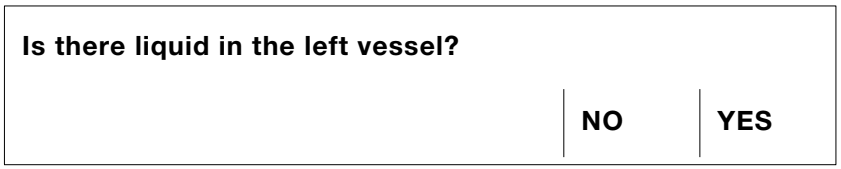
   Watch the liquid as it drains out of the left vessel. At the end of the liquid drain, a few small drops may flow back into the vessel.



5. Look at both transfer vessels. You should see liquid in the right transfer vessel and no more than a drop of liquid in the left transfer vessel. If you see liquid in the right vessel, press the ***Yes*** soft key.

   If there is no liquid in the right transfer vessel, press the ***No*** soft key.

   Again, watch the liquid as it drains out of the right vessel. At the end of the liquid drain, a few small drops may flow back into the vessel.



6. Again, check that there is liquid in only the left vessel and no more than a drop of liquid in the right vessel. If you see liquid in the left vessel, press the ***Yes*** soft key.

   If there is no liquid in the left transfer vessel, press the ***No*** soft key.

7. When this display appears, press the ***OK*** soft key to complete the flow test.

   If you have pressed the ***No*** soft key at any time during this flow test, the LCD displays the message ***Test failed***. Press the ***OK*** soft key.

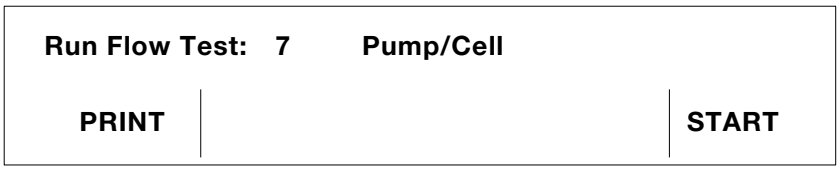
If Flow Test 6 fails:

- Check that the left and right transfer vessels are screwed on tightly. Check the two lines that go into each of the vessels for kinks or obstructions. There should be sufficient space (≈1 mm) between the open tips of these lines and the bottom of the vessels to allow unobstructed flow of liquid. After performing these checks, repeat Flow Test 6.
- If this flow test fails again, run Flow Test 3 to check DMF delivery (page 4-22), and run a Leak Self Test (page 4-13) to check for pressure leaks.
- If a pressure test failure occurs during this flow test, read ***What to do after a pressure test failure*** on page 7-12.

Flow Test 7: Pump/Cell

The "pump" in the name of this flow test refers to the configuration of transfer vessels that circulates liquids during synthesis. During this flow test, two conductivity readings are taken. The first one measures conductivity of DMF delivered to the flow test calibration tube. Next, a small amount of HBTU is delivered into the DMF solution and a second reading is taken. The first reading falls within the range of low conductivity readings, the second reading falls within the range of high readings.

1. To begin Flow Test 7, press the ***flow*** soft key in the Self Test Menu (Figure 4-6). Then press the *Next* key or press the numeric key "7" until ***Run Flow Test: 7*** appears on the LCD.



2. Press the ***START*** key. A two-minute procedure precedes the first conductivity reading.

3. Two conductivity measurements are taken during this test, with a two minute time lapse between each reading. When the conductivity value is within range, the LCD displays the conductivity. Press the ***OK*** key to continue the flow test after each display.

4. When this display appears, press the ***OK*** soft key to complete the flow test.

This message appears when a conductivity value is not within specifications. Press the ***OK*** key to see the value. Press the ***OK*** key again to continue Flow Test 7.

If the ***Test failed*** message appears at the end of Flow Test 7, repeat the flow test. If Flow Test 7 continues to fail, call Applied Biosystems Technical Support.

If a pressure test failure occurs during this flow test, read ***What to do after a pressure test failure*** on page 7-12.

Flow Test 8: Calibrate HBTU

WARNING **CHEMICAL HAZARD.** ***HBTU can cause allergic reactions and skin irritations in sensitive persons. Wear protective clothing and handle HBTU solution in a properly vented fume hood. See the MSDS in the Synergy User's Manual.***

This flow test measures delivery of HBTU (Peptide Synthesis Reagent A) to the left transfer vessel. To perform this flow test, you tare the left transfer vessel and then weigh the contents of the left vessel after each of three reagent deliveries.

1. To begin Flow Test 8, press the ***flow*** soft key in the Self Test Menu (Figure 4-6). Then press the *Next* key or press the numeric key "8" to make ***Run Flow Test: 8*** appear on the LCD.

Run Flow Test: 8	**CALIBRATE HBTU**	
PRINT		**START**

2. Press the ***START*** soft key to begin Flow Test 8.

Remove left vessel and put on scale.	
Zero scale then replace vessel	**OK**

3. Remove the left transfer vessel on the front of Synergy (Figure 4-15). To tare a scale, place the vessel on a scale that is accurate to ±1 mg and set the scale to zero.

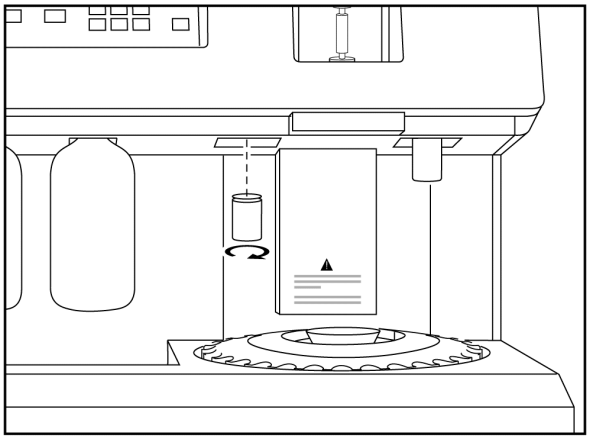


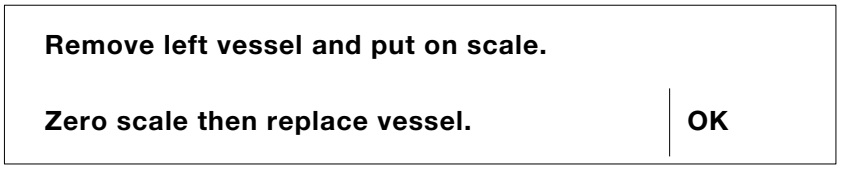
Figure 4-15. *Remove the left transfer vessel*

4. Replace the left transfer vessel on Synergy. Press the ***OK*** soft key.

Remove the left vessel and weigh.	**_ mg**
	OK

5. When this display appears, the left transfer vessel contains HBTU. Without spilling the contents, carefully remove the left vessel from the Synergy and weigh the HBTU delivered. Use the alphanumeric keys to enter this value in milligrams on the LCD. Press the ***OK*** soft key.

6. Replace the vessel on Synergy. Press the ***OK*** soft key.
7. When prompted, repeat Steps 5 and 6. The second HBTU weight should be greater than the first.



8. Again, remove the left transfer vessel (Figure 4-15), place it on a scale that is accurate to ±l mg, and set the scale to zero.
9. When prompted, repeat Steps 5 and 6 a third time.

10. When this display appears, the flow test has been successfully completed. Press the ***OK*** soft key to complete the flow test.

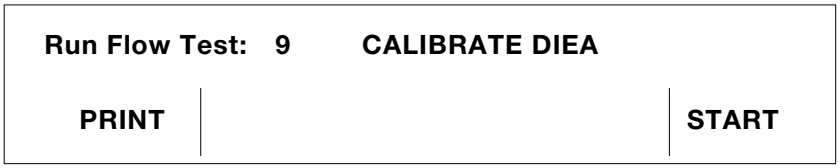
    If you see the message ***Test failed***, press the ***OK*** soft key and repeat the flow test. If the flow test continues to fail, call Applied Biosystems Technical Support.

Flow Test 9: Calibrate DIEA

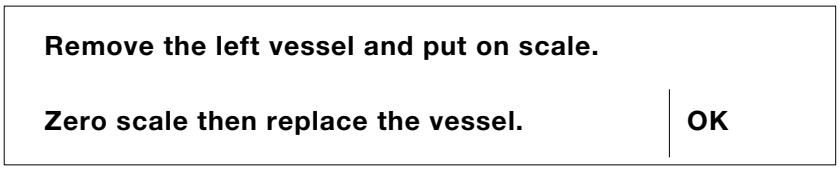
This flow test measures delivery of DIEA (Peptide Synthesis Reagent B) to the left vessel. As with Flow Test 8, you tare the left transfer vessel and then weigh the contents of the left vessel after each reagent delivery.

WARNING **CHEMICAL HAZARD*. DIEA (N, N-Diisopropylethylamine) can irritate skin, eyes and mucous membranes. Wear protective clothing and gloves when handling DIEA. See the MSDS in the Synergy User's Manual.***

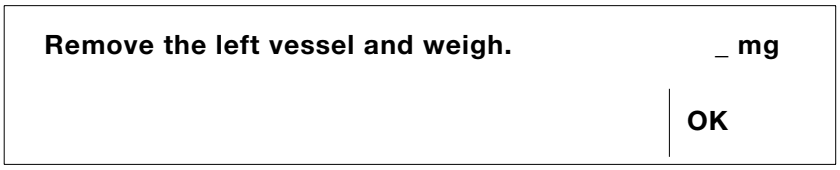
1. To begin Flow Test 9, press the ***flow*** soft key in the Self Test Menu (Figure 4-6 on page 4-8). Then press the *Next* key or press the numeric key "9" to make ***Run Flow Test: 9*** appear on the LCD.



2. Press the ***START*** soft key to begin Flow Test 9.

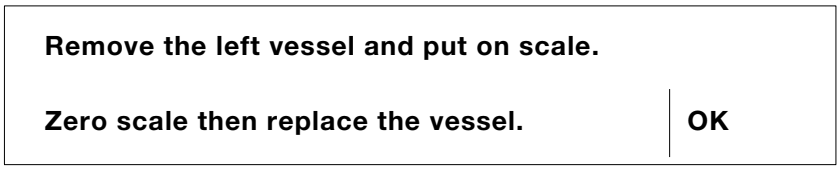


3. When this display appears, remove the left pump vessel on the front of Synergy (Figure 4-15 on page 4-28). Place the vessel on a scale that is accurate to ±1 mg and set the scale to zero.
4. Replace the left transfer vessel on Synergy. Press the ***OK*** soft key.



5. When this display appears, the left transfer vessel contains DIEA. Without spilling the contents, carefully remove and weigh the left vessel of DIEA. Use the numeric keys to enter this value on the LCD. Press the ***OK*** soft key.

6. Replace the left transfer vessel. Press the ***OK*** soft key.
7. When prompted, repeat steps 5 and 6. The second DIEA weight should be greater than the first.



8. Again, remove the left pump vessel, place it on a scale that is accurate to ±1 mg, and set the scale to zero.
9. When prompted, repeat Steps 5 and 6 a third time.

10. When this display appears, the flow test has been successfully completed. Press the ***OK*** soft key to complete this flow test.

    If you see the message ***Test failed***, press the ***OK*** soft key and repeat the flow test. If the flow test continues to fail, call Applied Biosystems Technical Support.

Cycle Test

During this test, you load a peptide synthesis column and 3 amino acid columns on to Synergy. The instrument then performs a pre-determined set of modules to synthesize LAGV, a test peptide. It takes approximately 3 hours to complete this test.

As synthesis progresses, the printer produces a trace of the deprotection and coupling reactions of the synthesis. Compare the features of the trace from your synthesis with the Trace Features described on page 2-16.

How to run the Cycle Test

1. Before running the Cycle Test, turn on the printer.
2. To begin the Cycle Self Test, press the ***cycle*** soft key in the Self Test Menu (Figure 4-6 on page 4-8).

```
Load the PSC-V

        AACs:     1 - G     2 - A   3 - L     START
```

3. Place a "V" (valine) peptide synthesis column (PSC) in the synthesis column position.

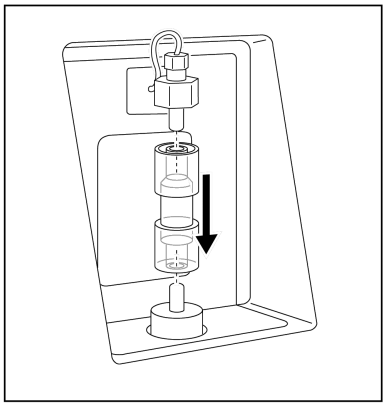


Figure 4-16. *How to place a PSC on the Synergy Peptide Synthesizer*

Twist and push the luer fittings on at both ends of the PSC. ***Do not unscrew the black plastic bushing attached to the top luer fitting.***

Caution	***Leaks at the peptide synthesis column position can damage the conductivity cell. To assure a leak-proof connection between the peptide synthesis column and the luer fittings, push the luer fittings onto both column ends with a slight twisting motion.***

4. Remove the amino acid column wheel and place the Amino Acid Column (ACC) labelled “G” in position 1, place the AAC labelled “A” in position 2, and place the AAC labelled “L” in position 3 (Figure 4-17).

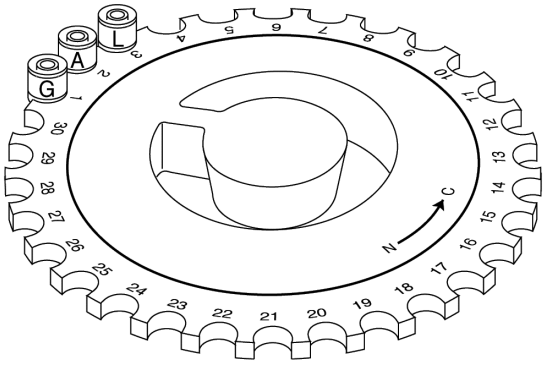


Figure 4-17. *Load the amino acid columns for the test peptide, LAGV.*

5. Replace the wheel. Press the ***START*** key to begin the cycle test.

   As the cycle test progresses, check around the PSC luer fittings for leaks. If you detect a leak at either end of the PSC, push and twist the end into its luer fitting. If a drop of liquid begins to form at the middle of the PSC, wick it away with a tissue. If the PSC continues to leak, terminate the synthesis (see ***How to terminate a synthesis*** on page 7-11) and restart the synthesis with a new PSC.

   It takes approximately 3 hours to complete this cycle test. During the test, the pre-programmed modules (Table 2-1 on page 2-5) direct the flow of reagents, monitor pressure, and generate a conductivity trace of the synthesis. You may leave the instrument unattended during this self test.

Run ended:	*13:58*	**Run began:**	*10:54*
Cycles run:	**3**		**OK**

6. This display appears when the synthesis is completed. Press the ***OK*** soft key.
7. Compare the Trace Features of the printout from your synthesis of LAGV to the example shown in Figure 2-7 on page 2-17. If any Trace Features are missing, call Applied Biosystems Technical Support.

   For descriptions of irregular traces, see ***Irregular Conductivity Traces*** on page 7-3.

Keyboard Self Test

Use the keyboard self test to check all the keys associated with the LCD (liquid crystal display) and the keyboard.

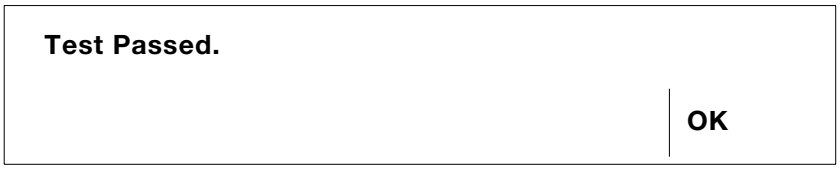
1. To begin the keyboard test, press the ***keyboard*** soft key in the Self Test Menu (Figure 4-6 on page 4-8).

2. Press each of the 5 soft keys under the LCD.

   As you press each key, the program emits a beep and the name of the key (***S1, S2***, etc.) disappears from the LCD.

3. Press the left and right arrow keys (← →) and each of the numeric keys, ***0-9***.
4. Press the keys labelled *Previous*, *Next*, and *Delete*.
5. Press the Menu key.

   If the keyboard is working properly, the next display reads:



6. Press the ***OK*** soft key.

   If the ***Test failed*** message appears on the LCD, call Applied Biosystems Technical Support.

Wheel Self Test

Use the Wheel Self Test to test operation of the autosampler jaws, the jaw sensors, and the mechanism that turns the amino acid column wheel. During this test, the wheel rotates to position one, where the jaws close down on a wheel test fixture.

WARNING **PERSONAL INJURY HAZARD.** ***The autosampler jaw is operated by a pneumatic valve under high pressure. Keep your fingers away from the jaws during this wheel test.***

1. To begin the Wheel Self Test, press the ***wheel*** soft key in the Self Test Menu.

Put a test fixture in	
position one on the wheel.	**START**

2. Place a wheel test fixture in position 1 (one) on the amino acid column wheel (Figure 4-18).

IMPORTANT *Place only one wheel test fixture on the wheel for this test!*

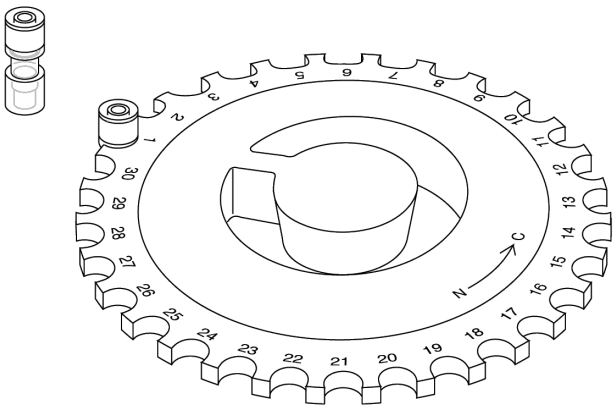


Figure 4-18. *Placing a wheel test fixture in position one on the wheel*

3. Place the wheel on top of the hub on the right side of Synergy. Slowly rotate the wheel until the protruding pin on the bottom of the wheel slips into the corresponding groove in the hub.
4. Press the ***START*** soft key.

   The wheel should now rotate so that the wheel test fixture in position one moves into the jaw assembly. Next, a series of tests check the motor and the jaw sensors.

   If any of these tests detect a mechanical failure, an error message appears on the screen. See ***Error Messages*** on page 7-20 for an explanation of error messages. Press the ***OK*** soft key and continue the Wheel Test to the end.

Test Passed.	
	OK

5. This display appears if the autosampler jaw and wheel are working.

Press the ***OK*** soft key to complete the Wheel Test.

If the message ***Test failed*** appears on the LCD, press the ***OK*** soft key and repeat the wheel test. If the Wheel Test continues to fail, call Applied Biosystems Technical Support.

Circuit Self Test

The Circuit Self Test rapidly checks the ROM (read only memory) card and the circuit connections to the valves.

To perform the Circuit Test, press the ***circuit*** soft key in the Self Test Menu (Figure 4-6 on page 4-8). The message ***Testing...*** appears on the LCD.

If the Circuit Self Test passes, press the ***OK*** soft key.

If the Circuit Self Test fails, one of the following messages appears:

- ROM memory error
- Valve continuity failure XXXXXXXXXXXXXXXXXXXX

The zeros and ones that appear (in place of the X's shown here) are a code that indicates which valves have caused the test failure. Make a note of the number code and call Applied Biosystems Technical Support.

5 Principles of Solid-Phase Peptide Synthesis on Synergy

Contents

Solid-Phase Peptide Synthesis

Solid-phase peptide synthesis (SPPS), originated by Merrifield,[1,2] involves the successive addition of amino acids to create a linear peptide chain. The C-terminus of the chain is covalently bound to a solid support, or resin, during synthesis. Three chemical reactions are repeated for each amino acid that is added to the growing peptide chain: deprotection, activation, and coupling.

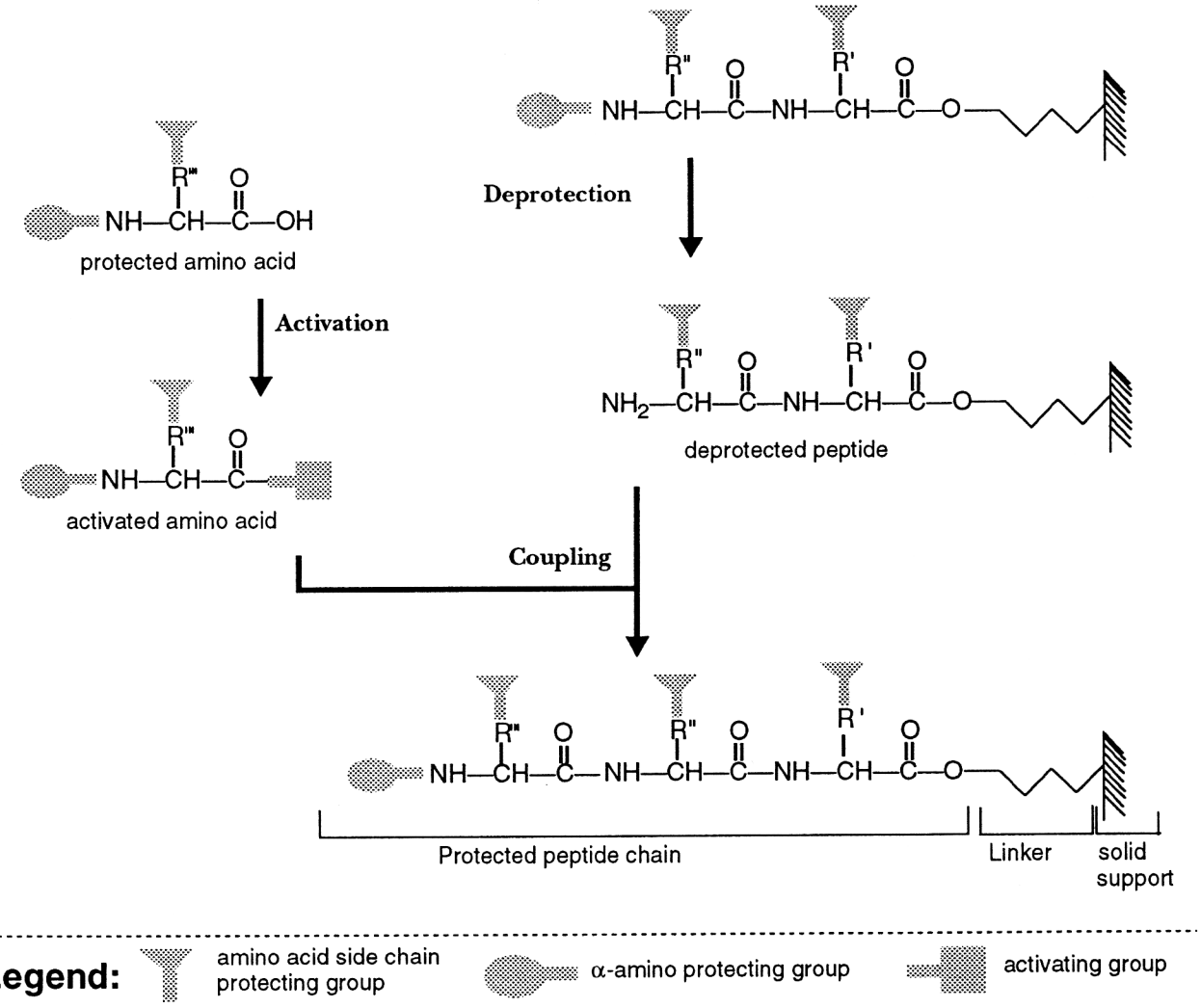


Figure 5-1. *Schematic representation of steps in solid-phase peptide synthesis*

1. Merrifield, R. B., 1963. "Solid phase peptide synthesis I. The synthesis of a tetrapeptide." *J. Am Chem. Soc.* 85:2149.

2. Merrifield, R.B., Stewart, J.M., and Jernberg, N., 1966. "Instrument for automated synthesis of peptides." *Anal. Chem*. 38:1905-1914.

Amino acid derivatives

The raw materials of SPPS are amino acid derivatives. During synthesis, chemical reactions can occur at three different locations on an amino acid: at the carboxyl group (COON), at the α-amino group (NH2), and at some side-chain functional groups (R). Amino acids are derivatized to prevent unwanted reactions at side-chain groups and to control reactions at the α-amino group. Derivatives used on Synergy have Fmoc protecting groups attached to the α-amino groups. The amino acids shown in Figure 5-1 also have side-chain protecting groups, though not all side chains have to be protected.

Deprotection

During *deprotection*, the Fmoc (9-fluorenylmethylcarbonyl) protecting group is removed to make the α-amino group on the peptide-resin accessible to a chemical reaction with an activated amino acid. As the Fmoc protecting group is removed, a conductive piperidine-carbamic acid salt is generated in the deprotection solution.[3]

Activation

Activation prepares the next amino acid to be added to the growing chain by converting its carboxyl group to an "active ester," a chemically reactive form.

Coupling

Coupling occurs when the activated amino acid forms an amide bond (CO-NH) with the α-amino group of the deprotected amino acid in the peptide.

Solid Support

The most widely used support for solid-phase peptide synthesis is a lightly cross-linked polystyrene resin in the form of tiny beads. On Synergy, the first step in every synthesis is the solvation of these beads with DME With solvation, the beads swell to several times their dry volume and form a solvated gel phase.

3. McFerran, N.V., Walker, B., McGurk, C.D., and Scott, F.C. 1991. Conductance measurements in solid-phase peptide synthesis. *Int. J. Pept. Protein Res.* 37:382-387.

The Changing Peptide-Resin Structure

As the peptide grows within the solvated gel matrix, its sequence influences the composition, conformation, and behavior of the resin beads. The conformation of the peptide-resin may effect the chemical reactivity of the syn thesis through the formation of inter- and intra-chain interactions.[4,5,6,7] Some peptide-resin structures may "bury" the growing N-terminus, which decreases reactivity. Inter-chain interactions may increase the effective crosslinking of the matrix, causing the structure to collapse and reducing the diffusion rates through the gel. Currently, we cannot predict when these sequence-dependent phenomena will occur.

Conductivity Monitoring

In each synthesis cycle, the Fmoc group is first removed by treatment with piperidine in DMF, generating a carbamate salt which can be detected by conductance monitoring. The rate at which this deprotection reaction proceeds serves as a valuable indicator of the peptide-resin reactivity. A rapid reaction gives rise to a sharp deptrotection spike; a slow reaction produces a broader peak.[8]

The conductivity cell on Synergy measures the conductive species every seconds. Synergy's controller records these conductivity values and every thirty seconds, averages these thirty values. As long as conductive species are being released at significant levels the controller continues to extend deprotec tion. The controller ends the deprotection reaction either when the difference between two consecutive 30-second value averages is equal to or less than 1 conductivity unit, or when thirty 30-second averages have been taken.

Peptide chemists at Applied Biosystems have observed that when a peptideresin requires extended deprotection times, the coupling time for the next amino acid should also be extended. For each deprotection, Synergy controller "remembers" the deprotection time and adjusts the subsequent coupling module. The resulting coupling

4. Bayer, E., and Goldhammer, C., 1992. Conformation-dependent coupling and deprotection: Diagnosis and cure. In *Peptides, Chemistry and Biology, Proceedings of the Twelfth American Peptide Symposium*, ed. J.A. Smith and J.E. Rivier. 589-590. ESCOM, Leiden.

5. Mutter, M., Altmann, K.-H., Belloff, D., Florsheimer, A., Herbert, J., Huber, M., Klein, B., Strauch, L., Vorherr, T., and Gremlich, H.-U., 1985. The impact of secondary structure formation in peptide synthesis. In *Peptides, Structure and Function, Proceedings of the Ninth American Peptide Symposium*, ed. C.M. Deber, V.J. Hruby, and K.D. Kopple. 397-405. Pierce Chemical Co., Rockford, III.

6. Kent, S.B.H., 1985. Difficult sequences in stepwise peptide synthesis:Common molecular origins in solution and solid phase, ibid., 407-414.

7. Live, D.H., and Kent, S.B.H., 1983. Correlation of coupling rates with physicochemical properties of resin-bound peptides in solid-phase synthesis. In *Peptides, Structure and Function, Proceedings of the Seventh American Peptide Symposium*, ed. V.J. Hruby, and D.H. Rich. 65-68. Pierce Chemical Co., Rockford, III.

8. Atherton, E., and Sheppard, R.C., 1985. Detection of problem sequences in solid-phase synthesis. In *Peptides, Structure and Function*, ed. C.M. Deber, V.J. Hruby, and K. D. Kopple. 415-418.

time is twice that of the deprotection to assure adequate coupling reaction for most syntheses.

Post-synthesis Cleavage and Side-Chain Deprotection

Once the synthesis is complete, you must remove, or *cleave*, the peptide from the solid support and *deprotect* the side-chain protecting groups on the peptide by treating the peptide-resin with a mixture of trifluoroacetic acid (TFA) and ion scavengers. Methyl-*t*-butyl ether (MTBE) can then be added to precipitate the peptide out of the cleavage mixture. This cleavage reaction procedure is detailed in Chapter 3 of this manual. After cleavage, the crude peptide can then be dissolved and lyophilized for storage.

The Synergy Synthesis Process

Synergy's peptide synthesis process incorporates traditional Fmoc[9] chemistry with HBTU [2-(1-H-benzotriazol-l-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] activation.[10,11] The three stages of SPPS—deprotection, activation, and coupling—are broken down into a series of pre-programmed modules or instrument operations, shown in Table 5-1.

Table 5-1. Pre-programmed Synergy Modules and Operations

Module	Operation
a	**A**ctivation
b	**B**egin synthesis
c	**C**oupling
d	**D**eprotection
e	**E**nd synthesis
f	**F**low—DMF delivery through PSC to waste
g	"**G**urgle"—solvents wash AAC and transfer vessels
h	alternate activation, for non-standard residues
i	**I**ncrement-moves the wheel to the next AAC position
j	**J**aws close on AAC and perform a leak test

Synthesis Columns and Reagents

Synergy **peptide synthesis columns** (PSCs) each contain 25 µmol of either pre-loaded resin or amide resin. Synergy **amino acid columns** (AACs) each contain 75 µmol amino acid, enough for a single coupling.

Each of the five bottled reagents on Synergy have specific applications in the peptide synthesis process.

DMF (N, N-dimethylformamide) is the primary solvent used in almost all stages of synthesis on Synergy. It solvates the peptide-resin at the beginning of synthesis; acts as a solvent for the piperidine deprotection reaction; is a cosolvent in activation with NMP (N-methylpyrrolidone) and DMSO (dimethyl sulfoxide); and washes the valve blocks and peptide synthesis columns after each coupling.

HBTU reagent, in combination with the **DIEA** (N,N-diisopropylethylamine) reagent are the activators. These reagents rapidly convert the carboxyl group to an "ac-

9. Carpino, L.A., and Han, G.Y. 1972. The 9-fluorenylmethoxycarbonyl amino-protecting group. *J. Org Chem.* 37:3404-3409.

10. Fields, C.G., Lloyd, D.H., Macdonald, R.L., Otteson, K.M., and Noble, R.L. 1991. HBTU activation for automated Fmoc solid-phase peptide synthesis. *Peptide Res.* 4:95-101.

11. Knorr, R., Trzeciak, A., Bannwarth, W., and Gillesen, D. 1989. New coupling reagents in peptide chemistry. *Tetrahedron Lett.* 30(15):1927-1930.

tive ester," a chemically reactive form that reacts with the deprotected α-amino group.

Piperidine, in combination with DMF, deprotects the Fmoc-protected (α-amino group on the peptide resin to prepare it for coupling.

THF (tetrahydrofuran) is the secondary solvent. It is both volatile and miscible with DME. At the end of synthesis, THF deliveries follow after DMF washes to displace the primary solvent. Pressurized gas delivery then removes THE, drying the contents of the peptide synthesis column.

Modules and Functions

Modules are composed of a series of steps. When all the steps in a module are performed, a defined action is accomplished. Some modules are flow tests; other modules perform routines within the larger process of peptide synthesis. Each step in a module is defined by one of the 99 software functions. Each function governs a particular action that is usually described in the function's name.

Some functions open or close Synergy's valves. For example, Function 10, GAS > TVB, opens valves 1, 7, and 12, to direct pressurized gas through the valves in the top valve block. Function #12, GAS > AAC, opens 5 valves to direct the flow of nitrogen gas to the amino acid column and out to waste.

Other functions contain directions for the Synergy controller. For example, when the controller reads Function 7, CHECK COND, the controller determines the average of 30 conductivity readings. The function takes no time to execute and appears only briefly on the LCD during a run. It has no effect on the valves.

"Toggle" functions come in pairs and turn a set of events or valves off and on. Pairs of toggle functions include the following: Function 86, +PRS PIP and Function 87, -PRS PIP, turn on and off the valves that control pressure on the piperidine bottle; Function 64, +PSC PATH, and Function 65, -PSC PATH, open and close valves to direct reagents through the peptide synthesis column; and Function 3, BEGIN LOOP, and Function 4, END LOOP, repeat a series of functions until a pre-determined condition is satisfied.

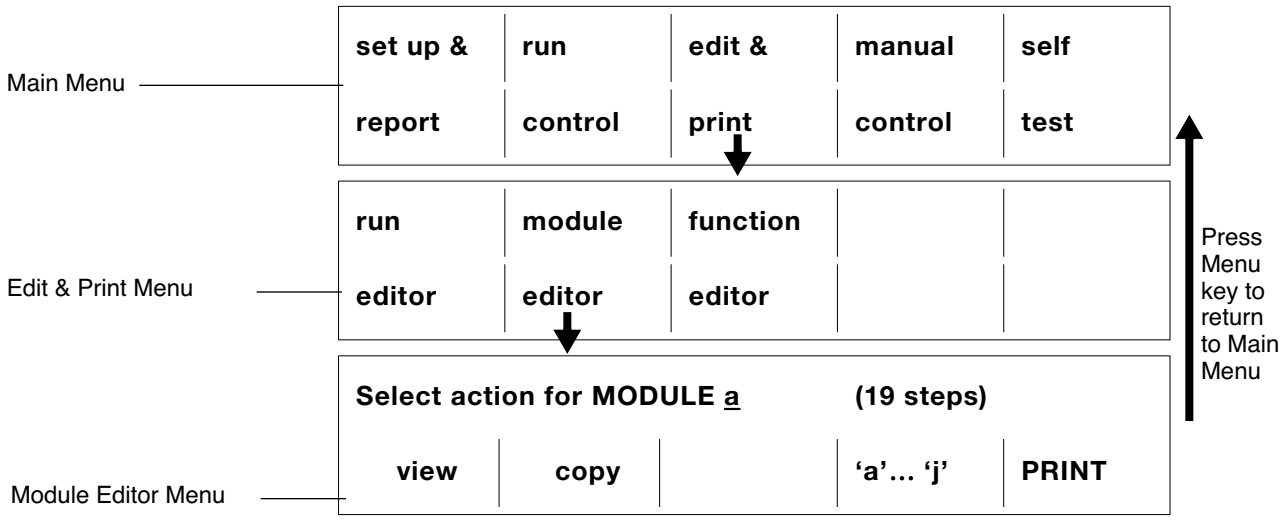
The following pages describe each module in the pre-programmed synthesis cycles with a modified plumbing schematic that shows reagent flow for some of the functions in these modules.

Table 5-2. Pre-programmed Synthesis Lines on Synergy

Line Name	Modules
BEG	b f
L (cycle line)	j d a c g f i
END	d e

You may print the steps of any module from the Module Editor Menu. See ***Annotated Module Printouts*** on page 5-18 for step-by-step descriptions of the synthesis procedures in each module. Table 5-3 summarizes the synthesis procedures that occur during each module.

How to print modules



1. Press the ***edit &print*** soft key in the Main Menu.
2. Press the ***module editor*** soft key in the Edit & Print Menu.

   In the Module Editor Menu, the cursor appears under a module name in the top line of the LCD.

   Pre-programmed synthesis modules are named a, b, c, d, e, f, g, h, i, and j. User-defined modules are given the upper-case letter names A, B, C, D, E, F, G, H, I, and J.
3. Press the *Previous* or *Next* keys to change the module letter. As an alternative, you may press the letter key for the module you wish to print.
4. When the top line of the LCD displays the desired module name, press the ***PRINT*** soft key.

Table 5-3. Stepwise Description of Procedures within each Pre-programmed Module

Module	Steps	Synthesis procedure
a	1-8	Measure activators (HBTU and DIEA)
	9-16	Cycle activators though AAC
	17-22	Begin transfer of activated amino acid to PSC
b	1-6	Pressure test PSC
	7-9	Position first AAC in jaws
	10-17	Solvate PSC resin and displace air from PSC
c	1-10	Cycle coupling solution through PSC for twice the time used to deprotect (module d, Step 5-9)
d	1-6	Pressure test PSC
	7-9	Pressurize PIP bottle and deliver DMF to PSC
	10	PIP and DM F flow through PSC for 60 seconds
	11 -14	Monitor conductivity of deprotection until change in conductivity in 30 seconds ≤ 1
	15-17	Depressurize PIP and wash PSC with DMF for 5 min
e	1-2	Wash PSC with DMF, 5 minutes
	3-4	Wash PSC with THF, 5 minutes
	5-8	Gas dry PSC, 10 minutes
f	1-6	Pressure test PSC
	7	Start delivery of DMF to PSC
	8-11	Monitor conductivity until change in conductivity in 30 seconds ≤ 1
	12	Stop delivery of DMF to PSC
g	1-2	Drain coupling solution to waste bottle
	3-8	Wash pump with 5 DMF deliveries and drain DMF wash though AAC to waste bottle
	9-11	Dry AAC
	12-16	Dry transfer vessels
i	1 -2	Open jaws and move wheel to next position
j	1-8	Close jaws and pressure test AAC
h	2	Interrupt synthesis for manual addition of non-standard residues
	3-10	Measure activators (HBTU and DIEA)
	11-14	Begin transfer of activated amino acid to PSC

The Synergy "Pump"

The system of pneumatically-operated left and right transfer vessels on Synergy acts as a pump that directs reagent flow throughout the activation and coupling steps of peptide synthesis. Pressurized gas and a series of valves deliver reagents through the pump to mix with the contents of either the peptide synthesis column or the amino acid column. Continuous delivery eliminates bubbles from the conductivity cell and enhances accurate conductivity monitoring in the peptide synthesis column (see

Conductivity Monitoring on page 5-5). This pumping system has fewer moving parts than a motor-driven pump and so is more simple, efficient, and reliable.

Illustrated Module Descriptions

Figure 5-2 is a simplified plumbing schematic of Synergy that shows the pump system, the conductivity cell, the valve blocks, and reagent bottles with the tubing that connects all these components. Pages 5-10 through 5-15 contain illustrations of some of the functions, to demonstrate how the pumping system and pressurized reagent deliveries perform parts of the synthesis process.

Caution ***The exterior panels on Synergy may be removed only by trained Applied Biosystems Service Personnel.***

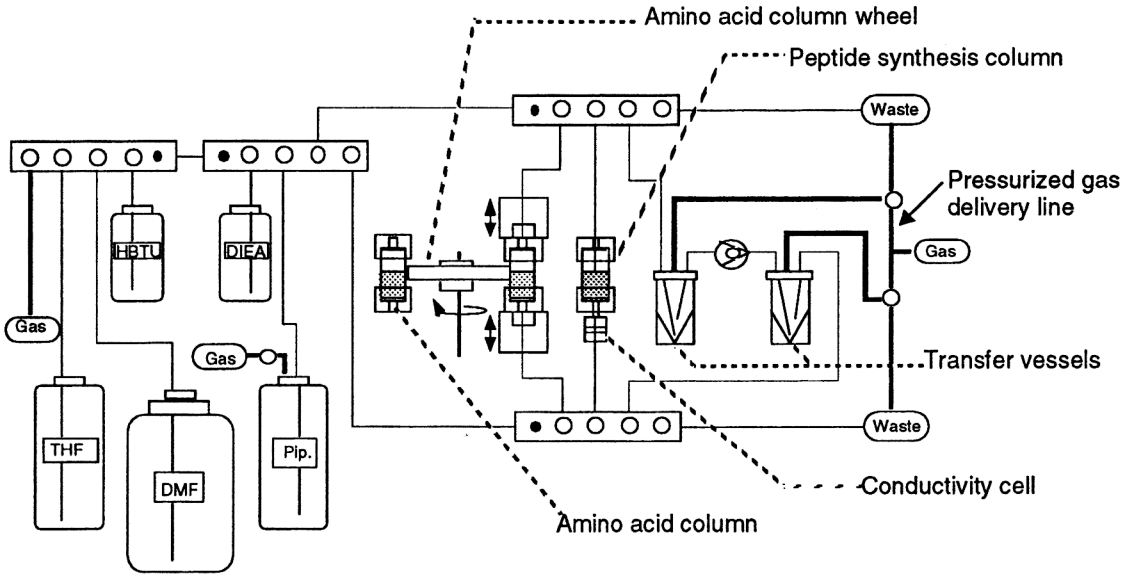
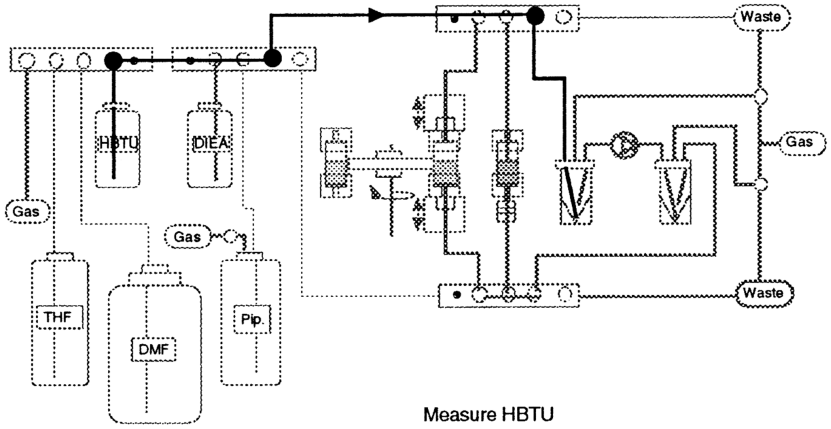


Figure 5-2. *Simplified Synergy Plumbing Schematic*

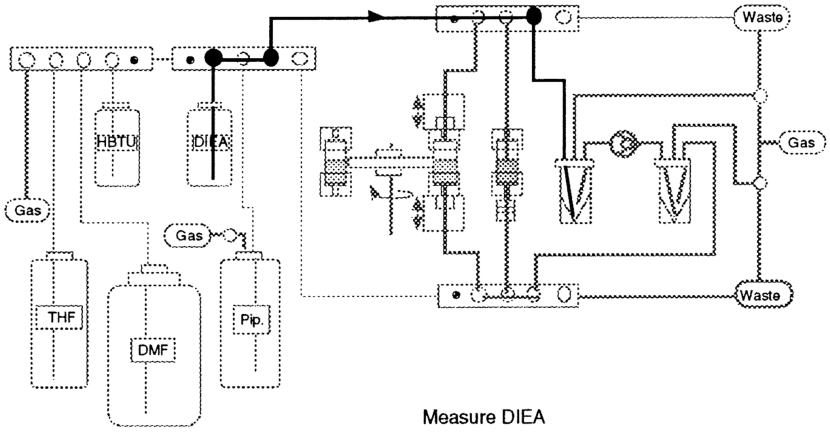
Module a: extraction and activation

Measured amounts of the activators, HBTU and DIEA, are cycled through the amino acid column (AAC). Activated amino acid begins transfer to the peptide synthesis column (PSC).

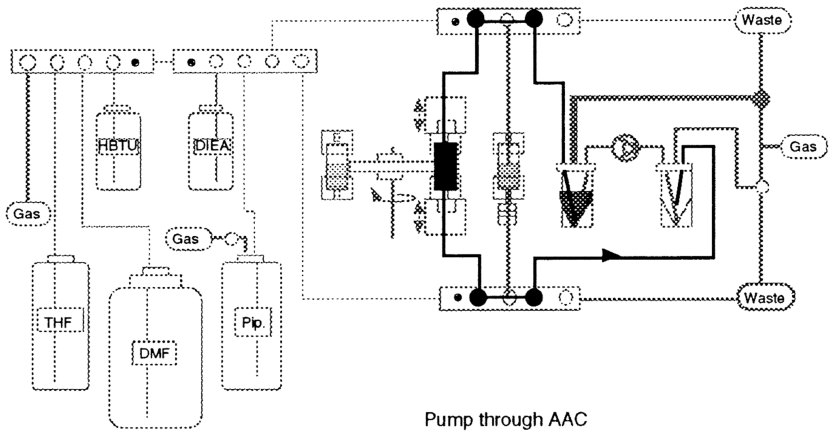
Function 45: Deliver the activator HBTU to the left transfer vessel



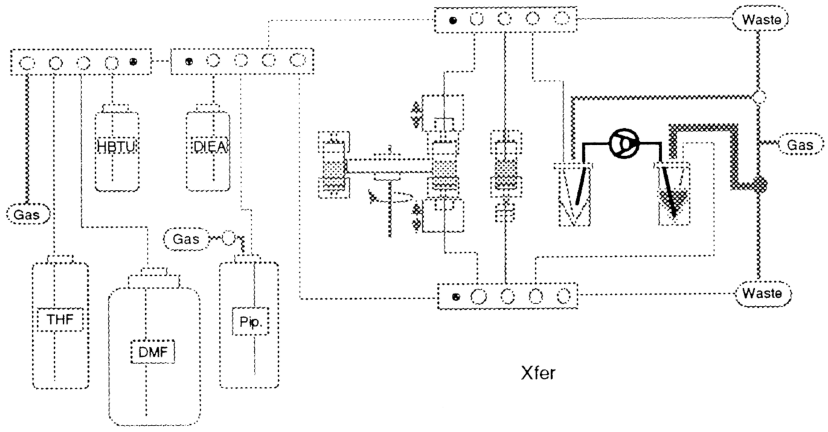
Function 46: Deliver the activator DIEA to the left transfer vessel



Function 60: PUMP Cycle activators from left transfer vessel, through the AAC, and to the right transfer vessel



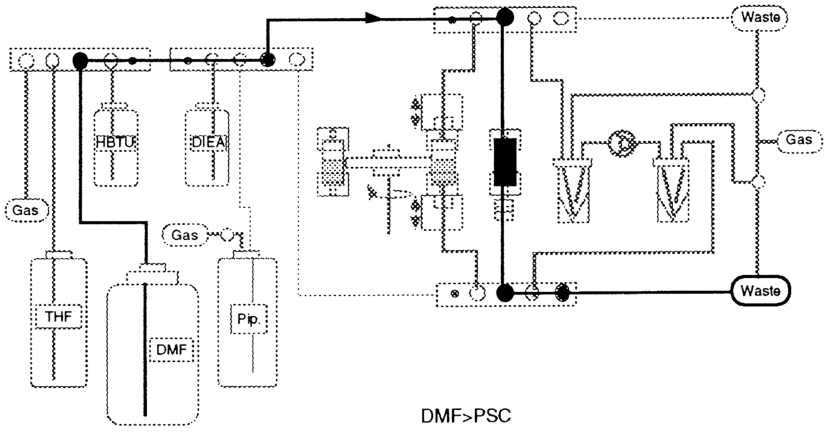
Function 61: Transfer the activated amino acid from the right transfer vessel, through the oneway valve, to the left transfer vessel



Module b: begin synthesis

PSC is pressurized to test for leaks and DMF is delivered to PSC to solvate the resin.

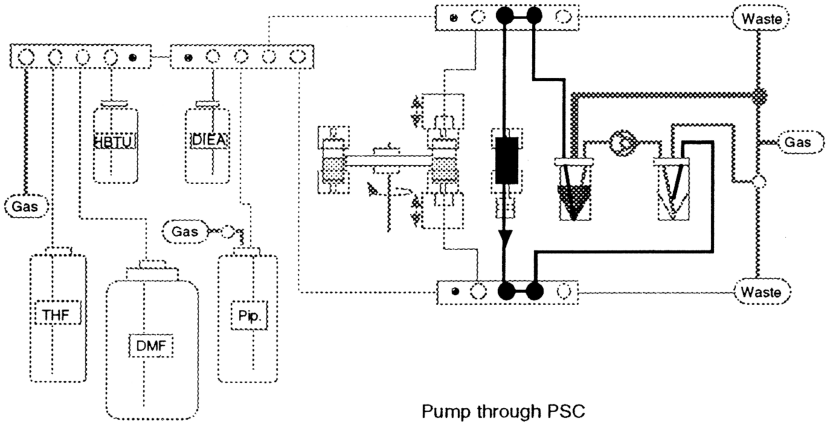
Function 33: Deliver DMF to the peptide synthesis column (PSC) to solvate the resin and displace air



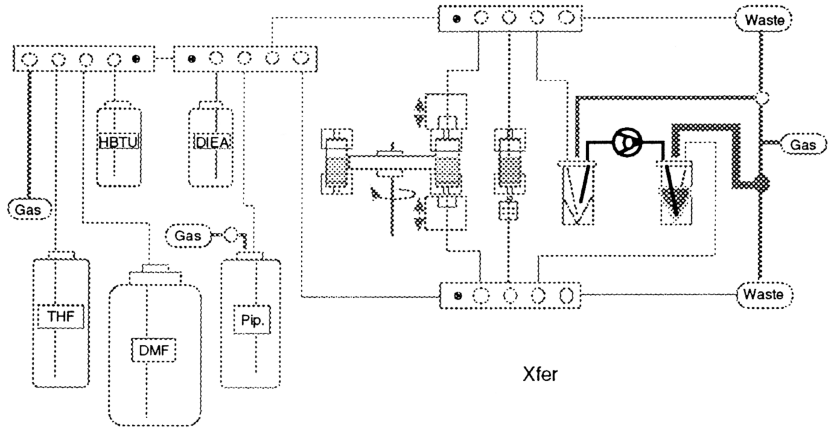
Module c: coupling

Coupling solution is cycled through the PSC for twice the amount of time used to complete deprotection in module d.

Function 60: PUMP coupling solution from the left transfer vessel, to the PSC, and then to the right transfer vessel



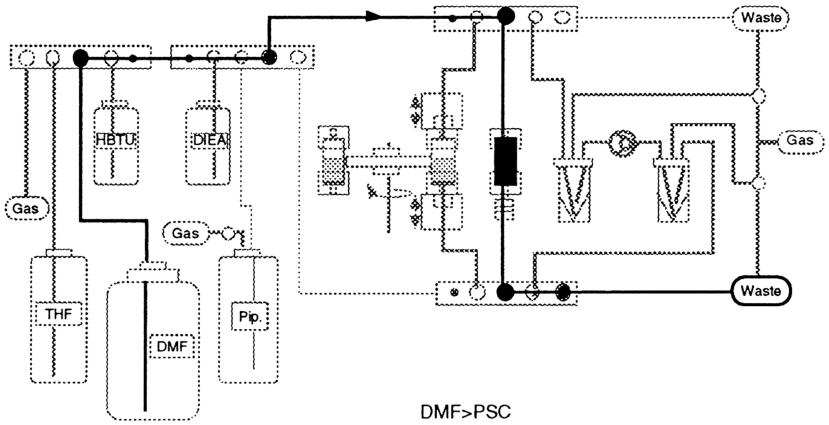
Function 61: Transfer the coupling solution from the right transfer vessel, through the oneway valve, to the left transfer vessel



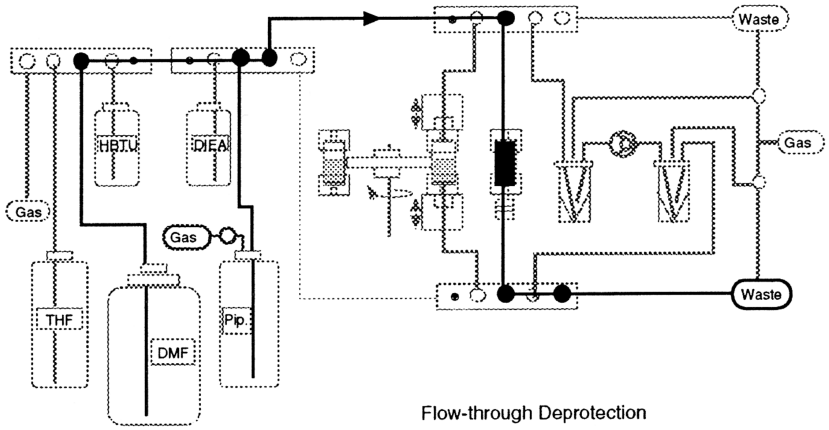
Module d: deprotection

Piperidine and DMF flow into PSC and, after one minute, conductivity monitoring begins. Conductivity readings are averaged every 30 seconds until the difference between two consecutive averages is ≤ 1conductivity unit.

Function 37: DMF delivery through the PSC



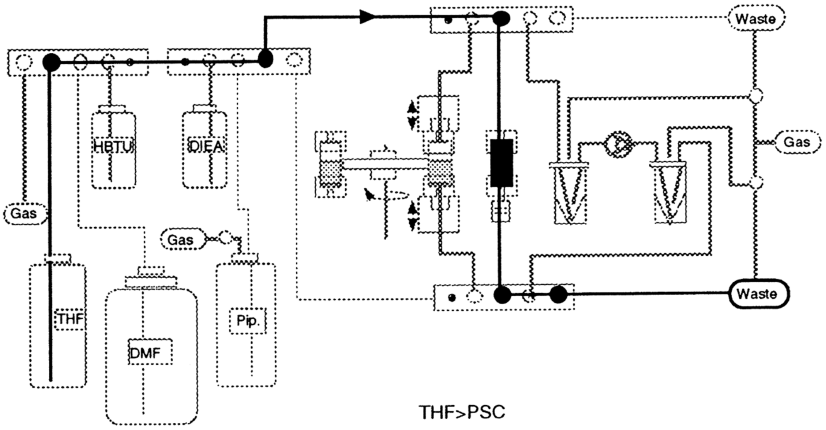
Function 40: Delivery of piperidine to the PSC



Module e: end synthesis

The PSC is washed with DMF for 5 minutes followed by THF for 5 minutes. Gas dries the contents of the PSC for 10 minutes.

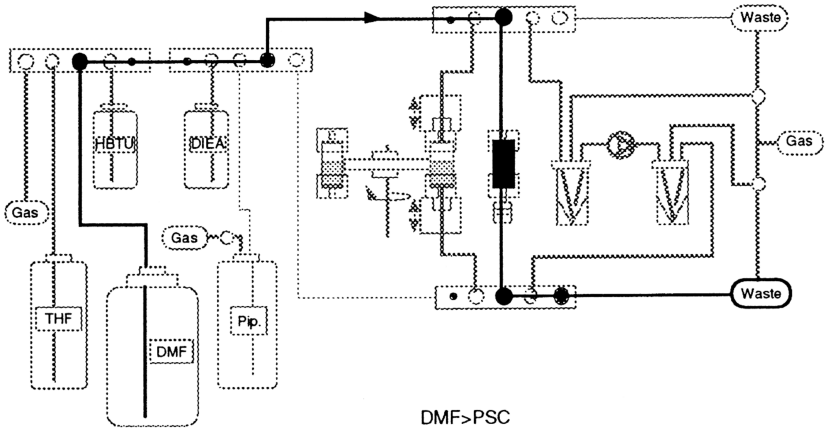
Function 23: THF deliverythrough the PSC



Module f: DMF flow to PSC

DMF flows into PSC and conductivity readings are averaged every 30 seconds until the difference between two consecutive averages is ≤ 1 conductivity unit.

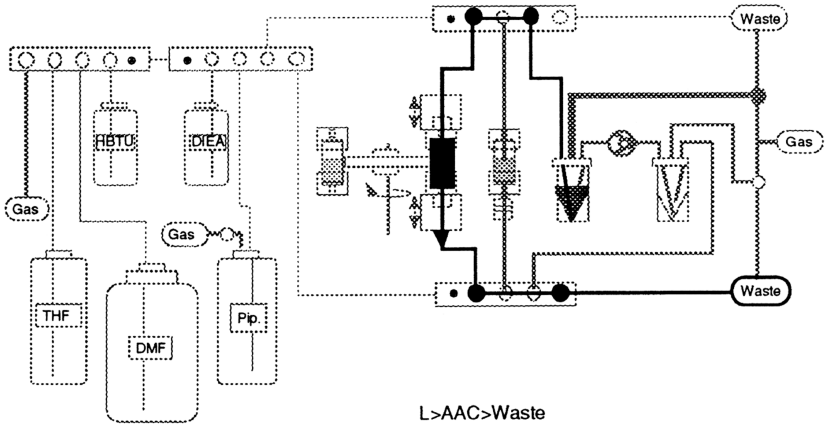
Function 37: Deliver DMF to the peptide synthesis column (PSC)



Module g: wash solvents through AAC and transfer vessels

Coupling solution flows into waste bottle. Transfer vessels and AAC are washed five times with DMF. DMF washes go to waste bottle. AAC and transfer vessels are gas-dried.

Function 66: Deliver DMF to the amino acid column (AAC) and out to waste



Module h: alternate activation

Similar to module a, this module also has a built-in interruption that allows you to add non-standard residues to the peptide. Chapter 6 gives procedures that incorporate module h.

Module i: incremental movement of amino acid column wheel

Autosampler jaws open and the amino acid column wheel moves counterclockwise, one position.

Module j: jaws close on AAC

Autosampler jaws close on amino acid column and a pressure test checks the system for leaks.

Annotated Module Printouts

The following pages show the steps and functions in each pre-programmed module with descriptions of the synthesis actions that occur in those steps.

In these module printouts, a few functions have an asterisk (*) after the time allowed for that function. The asterisk indicates the time allowed for that function is variable and dependent on a condition. For instance, in the printout for module a shown. here, Step 3: Function 45, HBTU >L, has a substitute time of 15 seconds. This value is dependent on the result of Flow Test 8 and may be different on your instrument. The default value for all substitute times is 0 (zero).

Module a: *extraction and activation*

Step#	Function #	Function Name	Time	*Action Description*
1	8	P/F TIME	2	
2	10	GAS > TVB	5	
3	45	HBTU > L	15*	
4	14	GAS > L	5	*Measure activators*
5	46	DIEA > L	10*	
6	14	GAS > L	5	
7	30	DMF > TVB	3	
8	35	DMF > L	3	
9	62	+ AAC PATH	1	
10	3	BEGIN LOOP	12	
11	60	PUMP	10	*Cycle activators through*
12	61	XFER	3	*the AAC*
13	4	END LOOP	1	
14	60	PUMP	60	
15	63	- AAC PATH	1	
16	61	XFER	7	
17	30	DMF > TVB	3	
18	31	DMF > BVB	3	
19	35	DMF > L	1	*Begin transfer to PSC*
20	9	P/F PAUSE	1	
21	8	P/F TIME	60	
22	67	L > PSC > WASTE	15	

* Substitute time

Module b: *begin synthesis*

Step#	Function #	Function Name	Time	*Action Description*
1	8	P/F TIME	60	
2	3	BEGIN LOOP	999	
3	84	PRS PSC	10	*Pressure test PSC*
4	88	+ PRS TEST	60	
5	89	- PRS TEST	5	
6	4	END LOOP	1	
7	53	OPEN JAWS	5	
8	51	HOME AAC	5	*Position first AAC*
9	52	CLOSE JAWS	5	
10	3	BEGIN LOOP	3	
11	31	DMF > BVB	3	
12	34	DMF > PSC (B)	30	*Solvate synthesis resin and*
13	1	WAIT	30	*displace air from PSC*
14	30	DMF > TVB	3	
15	33	DMF > PSC	30	
16	1	WAIT	30	
17	4	END LOOP	1	

Module c: *coupling*

Step#	Function#	Function Name	Time	*Action Description*
1	64	+ PSC PATH	1	
2	5	Begin loop	00*	
3	3	BEGIN LOOP	4	*Cycle coupling solution through*
4	60	PUMP	14	*the PSC for twice the*
5	65	- PSC PATH		*deprotection time*
6	61	XFER	2	
7	64	+ PSC PATH	1	
8	4	END LOOP	1	
9	6	End loop	1	
10	65	- PSC PATH	1	

* Substitute time

Module d: *deprotection*

Step#	Function #	Function Name	Time	*Action Description*
1	9	P/F PAUSE	1	
2	3	BEGIN LOOP	999	*Pressure test PSC*
3	84	PRS PSC	10	
4	88	+ PRS TEST	60	
5	89	- PRS TEST	1	
6	4	END LOOP	1	
7	30	DMF>TVB	60	
8	37	+ DMF > PSC	60	*Flow DMF through the PSC*
9	86	+ PRS PIP	1	*Pressurize the piperidine bottle*
10	40	+ PIP	60	*Add piperidine to DMF flow*
11	5	Begin Loop	30	
12	1	WAIT	30	*Wait until the conductivity*
13	7	CHECK COND	1	*Change ≤ 1 in 30 seconds*
14	6	End Loop	1	
15	87	- PRS PIP	1	
16	41	- PIP	300	*Turn off piperidine delivery and*
17	38	- DMF > PSC	1	*wash PSC with DMF for 5 min.*

Module e: *end synthesis*

Step#	Function #	Function Name	Time	*Action Description*
1	30	DMF > TVB	3	*Wash PSC with DMF, 5 minutes*
2	33	DMF > PSC	300	
3	20	THF > TVB	3	*Wash PSC with THF, 5 minutes*
4	23	THF > PSC	300	
5	10	GAS > TVB	3	*Gas dry PSC contents, 10 min.*
6	13	GAS > PSC	600	
7	10	GAS > TVB	10	
8	11	GAS > BVB	10	

Module f: *DMF flow to PSC*

Step#	Function #	Function Name	Time	*Action Description*
1	3	BEGIN LOOP	999	
2	84	PRS PSC	10	
3	88	+ PRS TEST	60	*Pressure test PSC*
4	89	- PRS TEST	1	
5	4	END LOOP	1	
6	30	DMF > TVB	3	
7	37	+ DMF > PSC	1	*Turn on DMF delivery to PSC*
8	5	Begin Loop	30	*Wait until conductivity change*
9	1	WAIT	30	*≤ 1 in 30 seconds*
10	7	CHECK COND	1	
11	6	End Loop	1	
12	38	- DMF > PSC	1	*Turn off DMF delivery to PSC*

Module g: *wash solvents through AAC and transfer vessels*

Step#	Function #	Function Name	Time	*Action Description*
1	52	CLOSE JAWS	6	*Close jaws on AAC*
2	68	L > WASTE	5	*Drain coupling solution to waste*
3	3	BEGIN LOOP	5	
4	36	DMF> R	5	*Wash pump with DMF and drain*
5	15	GAS > R	5	*to waste through the AAC, five*
6	61	XFER	7	*times*
7	66	L > ACC > WSTE	30	
8	4	END LOOP	1	
9	22	THF > AAC	3	*Dry AAC*
10	10	GAS > TVB	3	
11	12	GAS > AAC	120	
12	3	BEGIN LOOP	2	*Dry pumping vessels*
13	15	GAS > R	3	
14	61	XFER	4	
15	68	L > WASTE	3	
16	4	END LOOP	1	

Module i: *incremental movement of amino acid column wheel*

Step#	Function #	Function Name	Time	*Action Description*
1	53	OPEN JAWS	5	*Open jaws and move to next*
2	50	NEXT AAC	2	*AAC*

Module j: *jawsclose on AAC*

Step#	Function #	Function Name	Time	*Action Description*
1	3	BEGIN LOOP	999	
2	53	OPEN JAWS	5	
3	52	CLOSE JAWS	5	*Close jaws*
4	10	GAS > TVB	5	and
5	83	PRS AAC	10	*pressure test AAC*
6	88	+ PRS TEST	30	
7	69	- PRS TEST j	1	
8	4	END LOOP	1	

Module h: *alternate activation*

Step#	Function #	Function Name	Time	*Action Description*
1	8	P/F TIME	2	
2	2	INTERRUPT	1	
3	10	GAS > TVB	5	
4	45	HBTU > L	15*	
5	14	GAS > L	5	*Measure activators*
6	46	DIEA>L	10*	
7	14	GAS >L	5	
8	1	WAIT	60	
9	30	DMF > TVB	3	
10	31	DMF > BVB	3	
11	35	DMF > L	1	
12	9	P/F PAUSE	1	*Begin transfer to PSC*
13	8	P/F TIME	60	
14	67	L >PSC > WASTE	15	

* Substitute time

Recommended reading

In addition to the articles cited in the text of Chapter 5, the following books provide information on the development of solid-phase peptide synthesis.

Fields, G.B., and Noble, R.L., 1990. Solid-phase peptide synthesis utilizing 9-fluorenylmethoxycarbonyl amino acid. *Int. J. Peptide Protein Res.* 35:161-214.

Grant, G.A., ed. 1992. *Synthetic Peptides, A User's Guide*, W. H. Freeman and Company, New York.

Stewart, J.M., and Young, J.D. 1984. *Solid-phase Peptide Synthesis*, Second Edition, Pierce Chemical Company, Rockford, Illinois.

6 Advanced Operations

Contents

Editing Pre-Programmed Runs

You can synthesize most peptides with the pre-programmed modules built into Synergy operating software. Occasionally, you may want to edit the lines in the Run File to perform the following tasks:

- Extend coupling time.
- Double couple one or more amino acids in a sequence.
- Synthesize a peptide that contains a non-standard residue, such as a *d*-amino acid.
- Acetylate the N-terminal amino acid.
- Add residues to an existing peptide-resin.

In this chapter, we describe how to use the Run Editor Menu to perform these tasks.

The Run Editor Menu

A *run* contains all the module lines necessary to perform a complete peptide synthesis. A standard run consists of the following three lines:

- begin (b f) — to solvate the resin
- cycle line (j d a c g f i) — to couple residues
- end (d e) — to deprotect and dry the peptide-resin

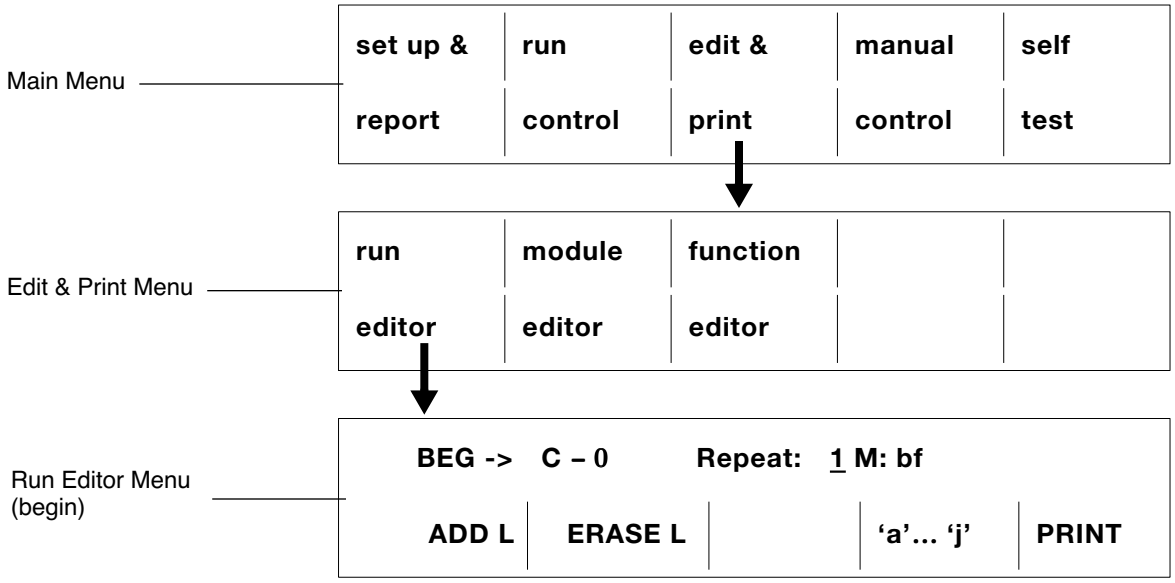


Figure 6-1. *How to go to the Run Editor Menu*

The Run Editor Menu has three parts or sub-menus: BEG (begin), L (cycle line) and END (end). Most of the time, you will probably edit on the cycle line.

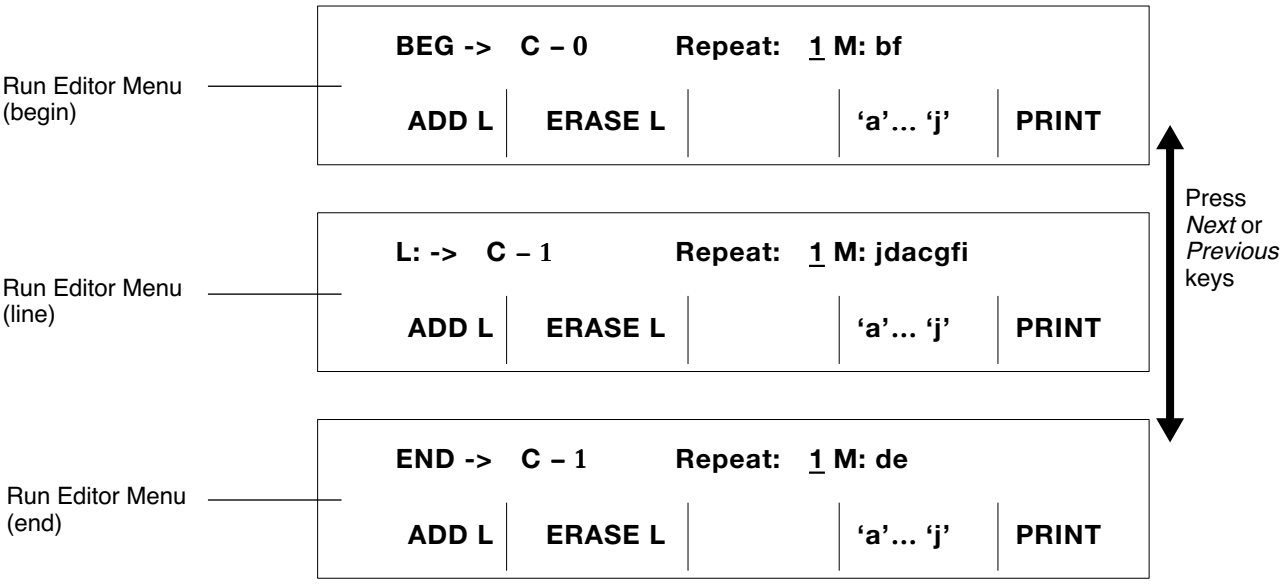


Figure 6-2. *Three parts of the Run Editor Menu*

How to print a Run File

The Run File contains all the lines shown in the Run Editor Menu. To print a Run File, press the ***PRINT*** soft key in the Run Editor Menu.

Figure 6-3 shows a printout of a Run File with the standard lines. When you first start up Synergy, the Run Editor contains this Run File.

Line#	Cycle #	Repeat#	Module List
Beg	0	1	bf
1	1	1	jdacgfi
End	1	1	de

Figure 6-3. *Standard Run File with the standard lines*

How to edit a run

1. In the Main Menu, press the ***edit & print*** soft key. In the Edit & Print Menu, press the ***run editor*** soft key (Figure 6-1).

2. Press the *Next* or *Previous* keys to go to the line you want to edit.

   If you want to add a new line to the run, go to the line sub-menu and press the ***ADD L*** soft key. The new line follows the current line.

3. Use the arrow keys to move the cursor to the right or left. Use the alphanumeric keys to enter letters or numbers.

   Press the ***'a'... 'j'*** soft key to toggle between lower-case (a, b, c) and upper-case (A, B, C) letters. Lower-case letter modules are pre-programmed, upper-case letter modules are user-defined.

   To remove a module from a line in the Run Editor menu, put the cursor under the module and press the Delete key.

   To insert a module in an existing line, put the cursor under the module immediately to the right of the intended insertion. When you press an alphanumeric key, the letter appears to the left of the cursor.

4. When you have finished editing in the Run Editor Menu, press the Menu key. The message on the LCD now asks if you want to save your changes.

5. Press the ***cancel*** soft key if you want to continue editing. Press the ***No*** soft key to get out of the Run Editor Menu **without** saving changes. Press the ***Yes*** soft key if you want to save the edited run.

How to erase a line in the Run Editor

1. In the Run Editor Menu, press the *Next* or *Previous* keys until the line you want to erase appears on the LCD.

2. Press the ***erase L*** soft key to delete the line from the run. Press the ***unerase L*** soft key to put an erased line back in the run.

How to start the synthesis after editing the run

Follow the procedure ***Start the Automated Synthesis on Synergy*** that begins on page 2-14. Start the synthesis in the Run Control Menu as you would any other synthesis.

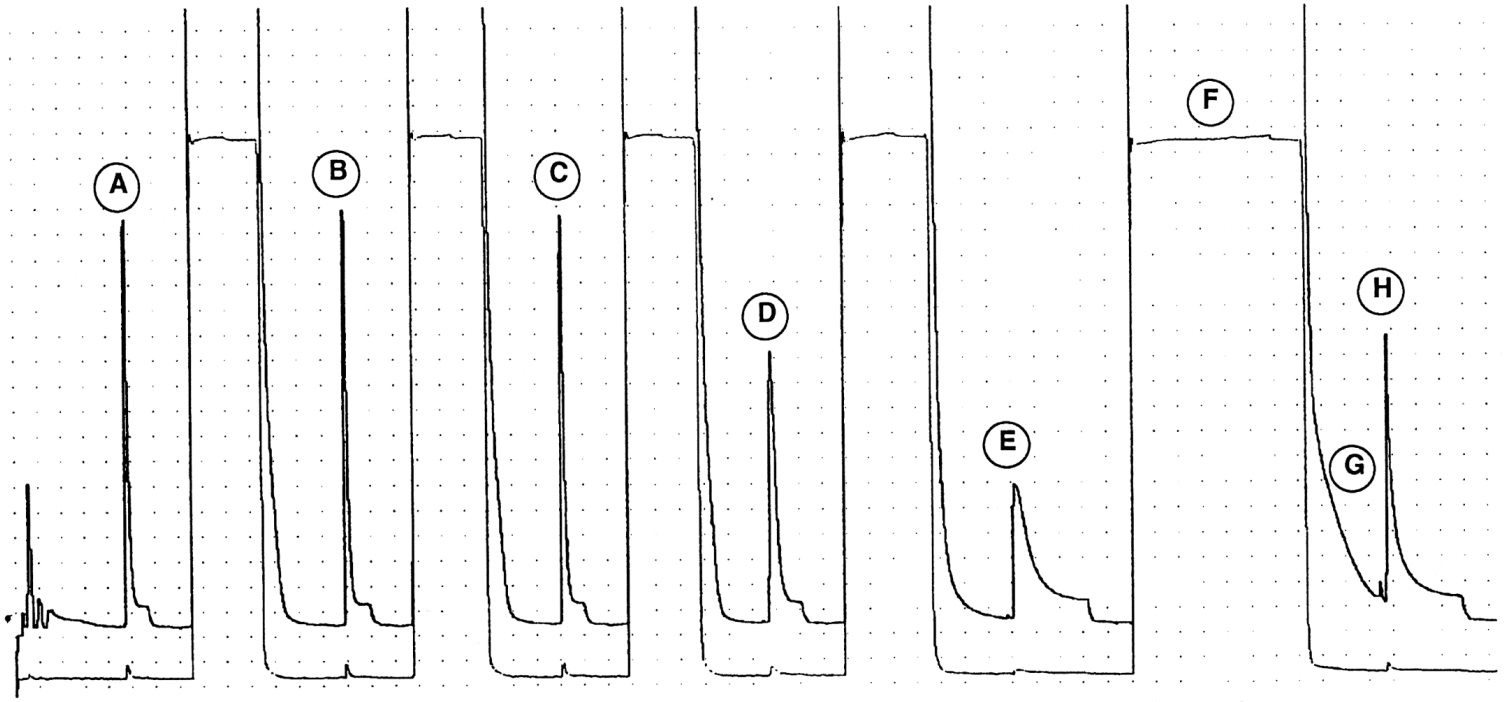


Figure 6-4. *Conductivity trace of a synthesis (scaled to fit page) with slow deprotections and an incomplete coupling*

Editing a Run to extend coupling time

The sequence of some peptides can result in slow reactions during a synthesis, as a result of the interactions within the peptide-resin and their influence on reagent diffusion rates (see ***The Changing Peptide-Resin Structure*** on page 5-5). Although it is difficult to predict which sequences will be difficult, conductivity monitoring can minimize their adverse effects.

Incomplete coupling. Figure 6-4 shows a conductivity trace with a series of two slow deprotections. With conductivity monitoring, the time Synergy allows for coupling is twice the time that was needed for deprotection. However, as Figure 6-4 demonstrates, even with a long coupling time at F, a slow, incomplete washout of reagents (G) followed the coupling, a strong indication that coupling was incomplete.

Deletion peptides. After an incomplete coupling or deprotection, the final crude product will contain a deletion peptide. A deletion peptide has one or more amino acids missing in its sequence and can often be detected by analysis with HPLC (high performance liquid chromatography).

Extended coupling time. If you know a particular sequence is difficult, you can extend the coupling time in the Run Editor before starting the synthesis. To extend coupling time, add an extra c (coupling) module to the cycle line for that coupling. For example, in Figure 6-4, there are four couplings preceding the incomplete coupling at E Therefore, you would add an extra c module to the second line (in this case, the fifth coupling) in the Run Editor.

IMPORTANT *Edited Run lines remain in the Run File until you return to the Run Editor and erase them.*

How to extend coupling time

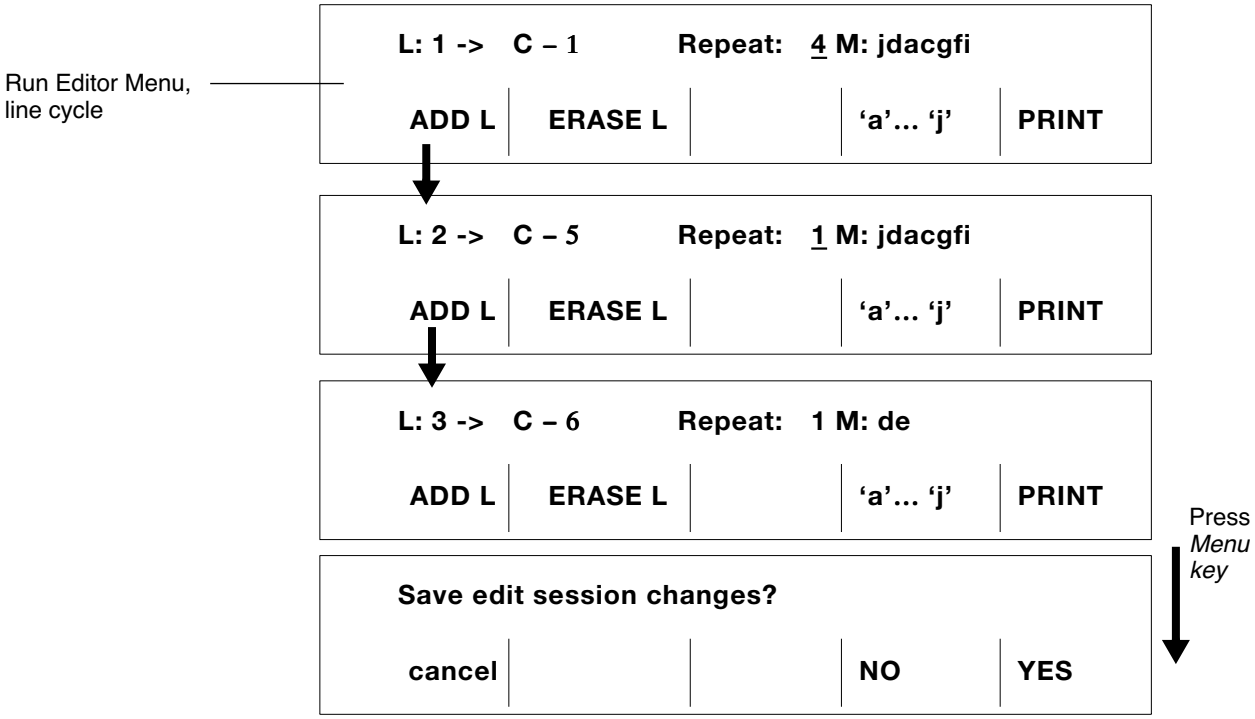


Figure 6-5. *Adding an extra c module to the second cycle line (fifth cycle) in a run*

1. In the Main Menu, press the ***edit & print*** soft key to go to the Edit & Print Menu, then press the ***run editor*** soft key. The LCD displays the Run Editor Menu with the Begin line.

2. Press the *Next* key to go to the Run Editor Menu that displays the cycle line (Figure 6-5).
3. After the word "Repeat" in the top line of the LCD, use the alpha numeric keys to enter the number of standard cycle lines that should precede the cycle line with extended coupling.

   For the example shown in Figure 6-4, there are four cycles before the coupling that must be extended. To have four standard cycles before the cycle with extended coupling, enter "4" after the word "Repeat."
4. Press the ***ADD L*** soft key.

   A new cycle line appears with "L: 2 -> C - 5" in the top line and the cursor after the word "Repeat:" (the second LCD in Figure 6-5).
5. Keep the default value, 1 (one), after the word Repeat. to indicate that this new line applies to only one cycle of deprotection and coupling. Then move the cursor to the space after the "M:" (module). Use the alpha-numeric keys to enter: "j d a c c g f i."

   "Line 2" now has two coupling modules in a row. Since each c module is twice as long as the deprotection module that precedes it, 2 c's extends coupling time to four times the deprotection time in that line.

Note ***The Synergy controller always repeats the last cycle line in any Run File until the jaw sensors detect an empty position on the amino acid column wheel.***

   If you do not want standard cycle lines to follow the extended coupling line, go to step 8 of this procedure.

   If you want standard cycle lines to follow the extended coupling line, you must add a third line in the Run Editor. Go on to step 6.
6. Press the ***ADD L*** soft key to add a third cycle line.

   A new line appears with "L 3 -> C-6" on the top line and the cursor after the word "Repeat."
7. Move the cursor to the space after the "M:" and enter: "j d a c g f i."

   If this is the last line cycle in the run, you do not have to enter a number after "Repeat." By default, the number 1 (one) appears after Repeat. The process described by this last line will be repeated until the jaw sensors detect an empty position on the amino acid column wheel.
8. Press the Menu key and save the edit session (see ***How to edit a run*** on page 6-4). Figure 6-3 shows the printed Run File for this example.
9. Follow the procedure ***Making a Peptide on Synergy*** on page 2-5. Start the synthesis in the Run Control Menu as you would any other synthesis.

Line#	Cycle #	Repeat#	Module List
Beg	0	1	bf
1	1	4	jdacgfi
2	5	1	jdaccgfi
3	6	1	jdacgfi
End	6	1	de

Figure 6-6. *Sample Run File with extended coupling time in the fifth cycle only*

IMPORTANT ***Edited Run lines remain in the Run File until you return to the Run Editor and delete them.***

Double Coupling

In most instances of incomplete coupling, extended coupling time may sufficiently improve synthesis. However in some cases, especially if the amino acid being coupled is an arginine, you should edit the line to a double coupling. With a double couple, two consecutive AACs on the wheel are activated in one cycle. This improves coupling by increasing the concentration of activated amino acid and extending coupling time.

Figure 6-8 shows the conductivity trace of a synthesis with incomplete couplings. Figure 6-9 shows the conductivity trace when the same peptide was synthesized with double couples at each site of difficult coupling.

How to program a double couple

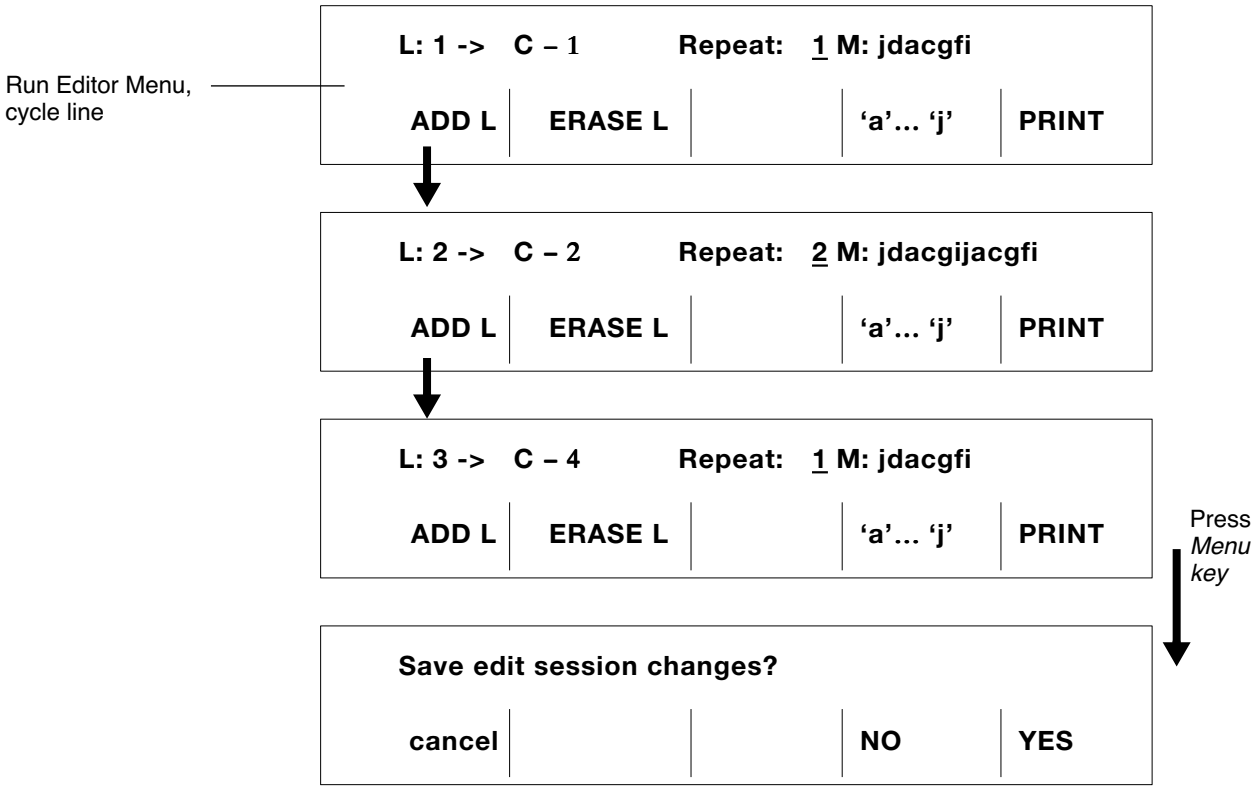


Figure 6-7. *How to program two, sequential, double couples*

1. Determine where the double couples should occur in the sequence. Place the appropriate AACs on the amino acid column wheel. You need two AACs of the same amino acid for each double couple.

   For example, assume in the sequence **Gln-Thr-Leu-Leu-Asn Arg-Gly-Ile**, we will double couple at both **Asn** and **Arg**. Synthesis on Synergy begins with the C-terminal amino acid, in this case **Ile**, in the PSC. **Gly** goes in position 1

on the AAC wheel. **Arg** and **Asn** both will be double coupled, so **Arg** goes in position 2 and 3, **Asn** goes in position 4 and 5.

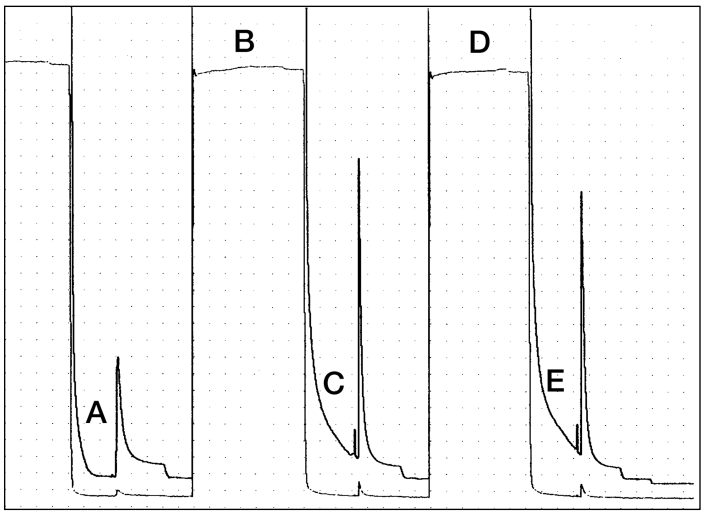


Figure 6-8. Conductivity trace, showing slow deprotections and incomplete couplings. *Coupling at B and D is followed by sluggish reagent washouts at C and E, without a return to the DMF baseline (A) within the maximum allotted time, an indication of incomplete coupling. Note the deprotection peaks after C and E are abnormally high, due to the presence of residual conductive material.*

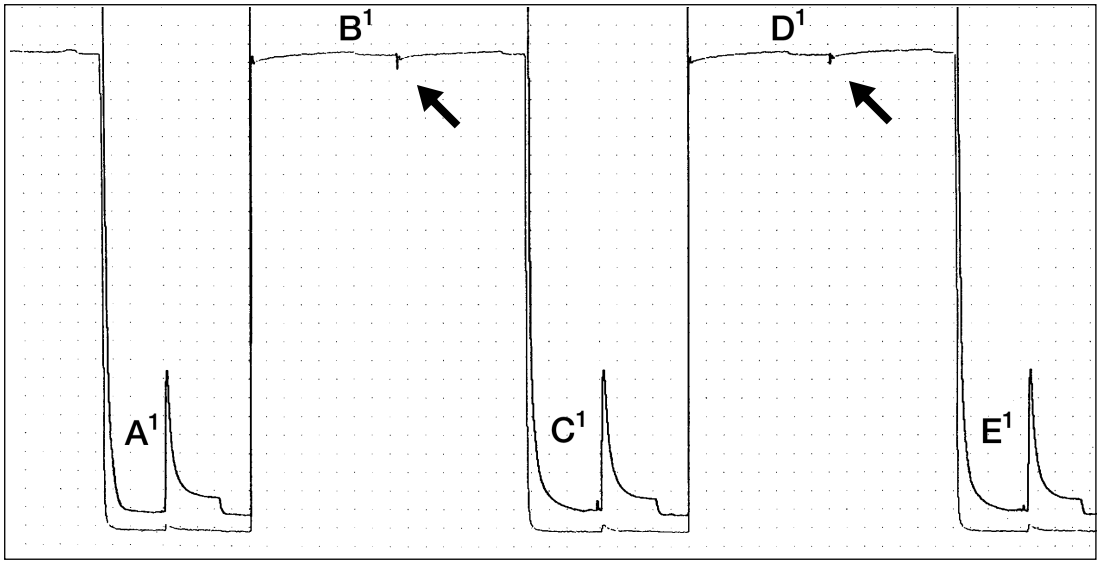


Figure 6-9. Conductivity trace of repeat synthesis of peptide in Figure 6-8. *This synthesis has a double couple at the sites of difficult coupling (B^1, D^1). Each coupling is over twice as long as its equivalent in the first trace and the reagent washouts at C^1 and E^1 show a more complete return to the DMF baseline. The arrows point to the beginning of the second coupling of the double couple.*

The rest of the AACs for the sequence follow on the wheel: **Leu** in 6, **Leu** in 7, **Thr** in 8, and **Gln** in 9.

2. Go to the Run Editor Menu, cycle line 1. Use the alpha numeric keys to enter how many times the standard line should be repeated.

   The standard cycle line can be applied to the first amino acid in the sequence, **Gly**. Line 1 (one) is used once, as the top LCD in Figure 6-7 demonstrates.

3. Press the ***ADD L*** soft key to enter the double couple line. After "Repeat:" enter the number of consecutive double couples.

   In this example, there are two double couples in a row, so a 2 appears after "Repeat:".

   If you want a double couple at every amino acid position until the end of the sequence, leave the Repeat value at one, the default value. The Synergy controller automatically repeats the last line in a run until the jaw sensors detect an empty position on the wheel.

4. After "M:" on the LCD for the second line, enter: j d a c g i j a c g f i.

5. Press the ***ADD L*** soft key to start line 3. Enter: j d a c g f i.

   In this example, we want standard cycle lines for all the amino acid couplings after the two double couple lines. We leave the default value 1 (one) after "Repeat." Figure 6-10 shows the Run File for this example.

Line#	Cycle #	Repeat#	Module List
Beg	0	1	bf
1	1	1	jdacgfi
2	2	2	jdacgijacgfi
3	4	1	jdacgfi
End	4	1	de

Figure 6-10. *Run File with a double couple in line 2.*

6. When you have finished editing the run, press the Menu key and save the editing session. Then continue with the procedure ***Making a Peptide on Synergy*** that begins on page 2-5. Start the synthesis in the Run Control Menu as you would any other synthesis.

IMPORTANT ***Edited Run lines remain in the Run File until you return to the Run Editor and delete them.***

Adding residues to a peptide-resin

Before you can add more amino acids to a peptide-resin, you must answer two questions:

1. Has the PSC been opened?
2. Is the Fmoc group attached to the peptide?

Has the PSC been opened?

PSCs (peptide synthesis columns) are sealed to prevent leaks that could damage Synergy. This seal is broken when the PSC is opened.

Caution	***Do not re-use PSCs that have been opened. Leaks from a PSC can damage Synergy.***

If the PSC has been opened, put 25 µmol of the peptide-resin in a new, empty, fritted PSC. Tightly press the top and bottom parts of the PSC together. During the begin line, Synergy runs a pressure test to check for a leak in the PSC.

If the PSC has not been opened, use it as is.

Is the Fmoc group still on the peptide?

If you used the standard End line (d e) to synthesize the peptide-resin, the Fmoc group has been removed from the peptide.

If you **do no**t use the End line at all when you synthesize the peptide, the peptide-resin is still solvated and the contents of the PSC are still wet with DMF at the end of the last cycle. **Do not remove the PSC from Synergy without first running module e**.

WARNING	**CHEMICAL HAZARD*. DMF is flammable and an irritant to skin and eyes. Avoid all skin contact with and inhalation of DMF.***

To keep the Fmoc group on the peptide-resin, remove the d module from the END line in the Run Editor. The End line now contains only module e.

Line#	Cycle #	Repeat#	Module List
Beg	0	1	bf
1	1	1	jdacgfi
End	1	1	e

Figure 6-11. *Run File with End modified to keep the Fmoc group on the peptide-resin*

How to add more amino acids to a dry peptide-resin WITHOUT the Fmoc group

1. Place the PSC that contains the peptide in the PSC position on Synergy.
2. Place the appropriate AACs on the amino acid column wheel. As with a routine synthesis, remember to put the AAC nearest the C-terminal end in position 1.
3. Go to the Run Editor Menu and edit Line 1 to read: j a c c c g f i.

   The d module is not needed here because the Fmoc group has already been removed. Extra c modules extend coupling time to compensate for the missing d module.
4. After editing Line 1, press the ***ADD L*** soft key. Enter the standard cycle modules on Line 2: j d a c g f i. By default, a 1 (one) appears after the word "Repeat:". Figure 6-3 shows the Run File for this example.

Line#	Cycle #	Repeat#	Module List
Beg	0	1	bf
1	1	1	jacccgfi
2	2	1	jdacgfi
End	2	1	de

Figure 6-12. *Run File for adding amino acids to peptide-resin without Fmoc group*

5. Follow the procedure ***Start the Automated Synthesis on Synergy*** that begins on page 2-14. Start the synthesis in the Run Control Menu as you would any other synthesis.

IMPORTANT *Edited Run lines remain in the Run File until you return to the Run Editor and delete them.*

How to add more amino acids to a dry peptide-resin WITH an Fmoc group

1. Place the PSC that contains the peptide-resin in the PSC position on Synergy.
2. Place the appropriate AACs on the amino acid column wheel. As with a routine synthesis, remember to put the AAC nearest the C-terminal end in position 1.

Line#	Cycle #	Repeat#	Module List
Beg	0	1	bf
1	1	1	jdacgfi
End	1	1	de

Figure 6-13. *Run File for adding amino acids to peptide-resin with an Fmoc group*

3. Follow the procedure ***Start the Automated Synthesis on Synergy*** that begins on page 2-14. Use the standard lines in the Run. Start the synthesis in the Run Control Menu as you would any other synthesis.

Module h

Module h is a pre-programmed module that can be used as an alternative to module a (activation). Use module h to add *d*-forms of an amino acid or a non-standard Fmoc-amino acid—such as ornithine or-norleucine—in the middle of a peptide, or to add an organic acid—such as acetic acid or biotin—to the end of a peptide. You can use module h to activate the compound on Synergy, or to add pre-activated compounds.

How to use module h to activate non-standard amino acids on Synergy

1. Assemble the amino acid columns for the synthesis and determine where the non-standard residue will be added in the peptide. Put a calibration column or a used AAC in the wheel position for the nonstandard residue.

IMPORTANT ***Do not leave any empty positions on the wheel between the AAC in position 1 and the N-terminal AAC. Place a calibration column or a used AAC in the wheel position for the non-standard residue.***

   For example, if you want to acetylate the N-terminal amino acid of a nonamer, the acetyl group is added after the ninth amino acid in the sequence. Assuming there are no double couples in the sequence, the first nine cycles of the run can use the standard line of modules.

2. In the Run Editor Menu, enter the number of times the standard cycle line should be repeated. For our example, you would enter 9 after "Repeat."
3. Press the ***ADD L*** soft key to add a line that will activate and couple the compound. In our example, the compound will be added to the N-terminal amino acid.
4. In cycle line 2, enter the modules: j d h c g f i.

   If the non-standard residue is being added in the middle of the peptide, you must press the ***ADD L*** soft key to add another cycle line, in this case line 3, that will contain the standard activation module, module a.
5. Press the *Next* key to go to the End line. Move the cursor under the d module and press the delete key.

Line#	Cycle #	Repeat#	Module List
Beg	0	1	bf
1	1	8	jdacgfi
2	9	1	jdhcgfi
End	9	1	e

Figure 6-14. *Sample Run File for N-acetylating a 9-residue peptide*

6. Continue with the procedure ***Making a Peptide on Synergy*** that begins on page 2-5.

   Start the synthesis in the Run Control Menu as you would any other synthesis. When the synthesis reaches Step 1 of module h, Synergy automatically pauses.

7. When the interruption occurs during module h, remove the left transfer vessel (Figure 6-15).

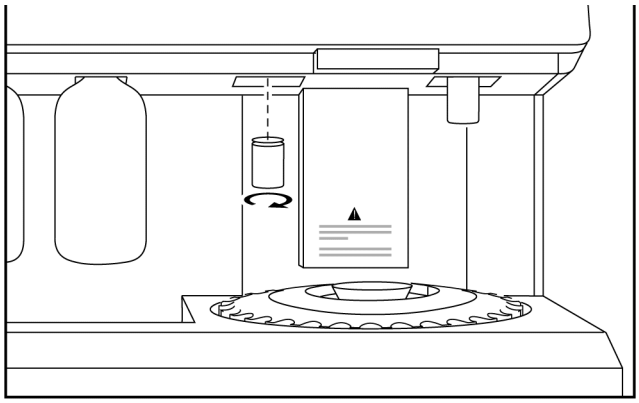


Figure 6-15. *Removing the left transfer vessel*

8. Dissolve 75 µmol of the non-standard residue in 300 µL solvent.

   Most compounds will dissolve in DMF. If 75 µmol of the compound does not dissolve in 300 µL DMF, you may add up to 100 µL DMSO to encourage solvation. Or add 75 µmol of the compound to 300 µL of a 1:1 solution of NMP:DMSO.

Caution	***Undissolved particulate matter may damage the valves on Synergy. Do not put cloudy solutions or undissolved material in the left transfer vessel.***

If the solution of pre-activated compound in DMF is cloudy or contains undissolved material, filter the solution. A suitable filter may be prepared by pushing a small piece of cotton or glass wool into a disposable Pasteur pipette, as shown in Figure 6-16.

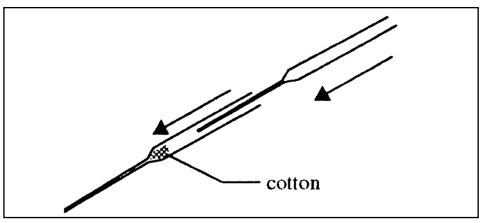


Figure 6-16. *Prepare a filter from a disposable pipette*

9. Use a pipette to transfer the resulting solution to the left transfer vessel. Screw the left vessel tightly in place.
10. Press the ***PAUSE**** soft key to continue synthesis.

How to use module h to add a pre-activated compound

1. Follow the previous procedure through step 7.
2. Dissolve 75 µmol of the pre-activated compound in 1.5 mL DMF.

Caution	***Undissolved particulate matter may damage the valves on Synergy. Do not put cloudy solutions or undissolved material in the left transfer vessel.***

   If the solution of pre-activated compound in DMF is cloudy or contains undissolved material, filter the solution. A suitable filter may be prepared by pushing a small piece of cotton or glass wool into a disposable Pasteur pipette, as shown in Figure 6-16.

   Use a pipette to transfer the resulting solution to the left transfer vessel. Screw the left vessel tightly in place.
3. In the Run Monitor, press the ***jump S*** soft key.
4. In the Jump Step Menu, enter "9" after "S:" to jump to step 9 in module h. This jump bypasses the steps that direct activator deliveries.

Caution	***If you do not perform steps 4, 5, and 6 of this procedure, activators may react with the peptide-resin and partially cap the peptide, essentially terminating the synthesis.***

Module h: alternate activation

Step#	Function #	Function Name	Time	Action Description
1	8	P/F TIME	2	
2	2	INTERRUPT	1	
3	10	GAS > TVB	5	Measure activators
4	45	HBTU > L	15*	
5	14	GAS >L	5	
6	46	DIEA > L	10*	
7	14	GAS > L	5	
8	1	WAIT	60	
9	30	DMF > TVB	3	
10	31	DMF > BVB	3	
11	35	DMF > L	1	Begin transfer to PSC
12	9	P/F PAUSE	1	
13	8	P/F TIME	60	
14	67	L >PSC > WASTE	15	

* Substitute time

Jump from interrupt in module h to Step 9

Figure 6-17. *Jumping over activator delivery steps in module h*

5. Press the ***JUMP*** soft key to jump to the step 9 and return to the Run Monitor.
6. Press the ***PAUSE**** soft key to continue synthesis.

The Module Editor Menu

A module contains the steps needed to perform a particular process within a synthesis. All the pre-programmed synthesis modules have **lower-case** letter names, such as a, b, c, etc. See ***Annotated Module Printouts*** on page 5-18 for printouts of all the pre-programmed synthesis modules. The pre-programmed modules may be viewed or copied, but they cannot be edited.

In the Module Editor Menu, you may edit any module that has an **upper-case** letter name, such as A, B, C, etc. To edit one of the pre-programmed modules, you must first copy all its steps into a module with an upper-case letter name. Modules with upper-case letter names are called user-defined modules.

How to copy a module

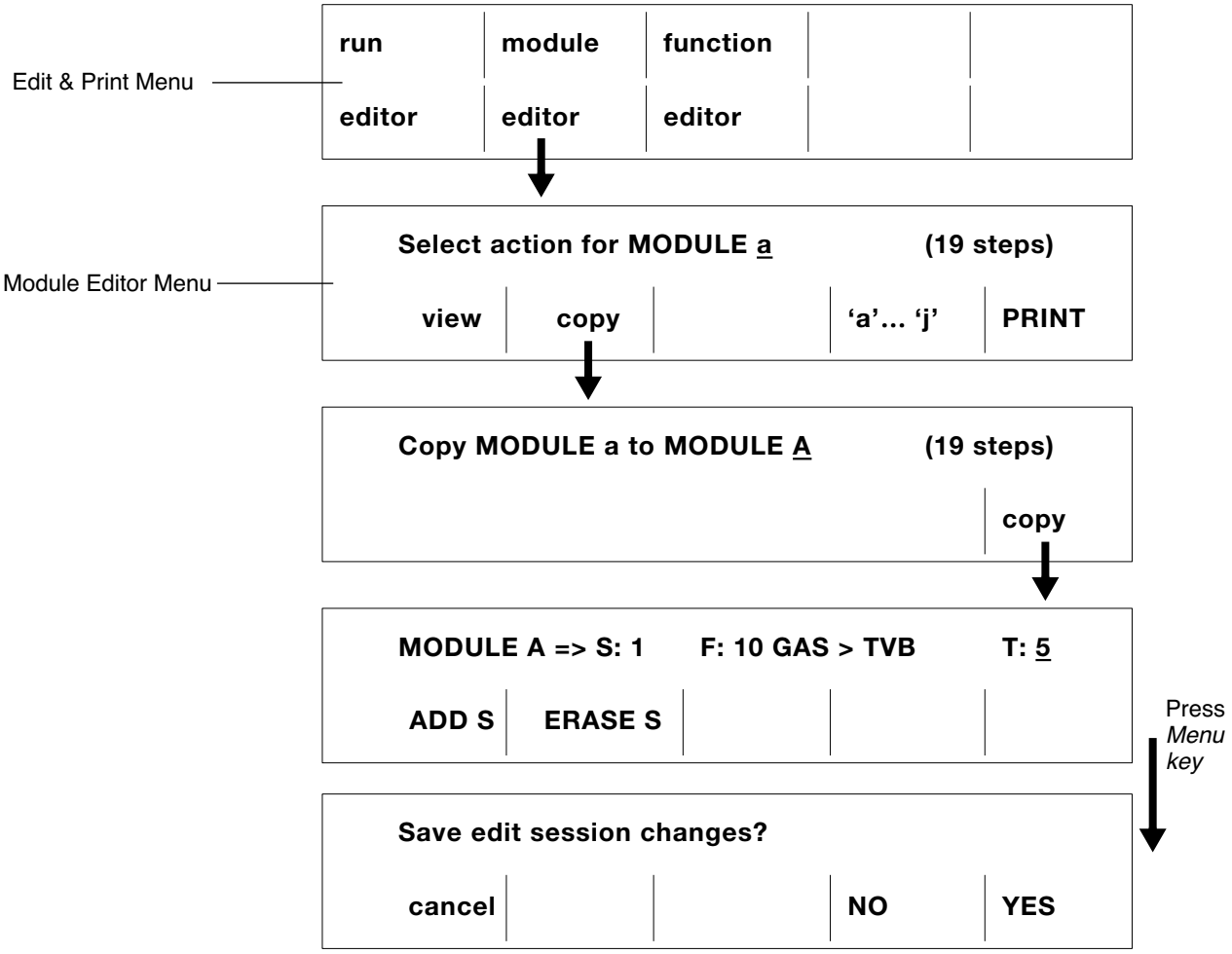


Figure 6-18. *How to copy a module*

1. In the Main Menu, press the ***edit & print*** soft key. In the Edit & Print Menu, press the ***module editor*** soft key.
2. In the Module Editor menu (Figure 6-18), use the alphanumeric keys to select the module you wish to copy. The letter name of the module appears after the words "Select action for MODULE."

   Press the ***'a'... 'j'*** soft key to toggle between lower-case letters and upper-case letters..
3. Press the ***copy*** soft key to copy the selected module into a module with an upper-case letter name. Use the alphanumeric keys to select an upper-case module.

   In the example shown in Figure 6-18, module a will be copied into module A.
4. Press the ***copy*** soft key to complete the copy.

   The LCD now displays the new module. You can press the *Next* and *Previous* keys to see the steps displayed one at a time. You can also add, erase, and "unerase," (bring back steps that were just erased) in this menu.
5. Press the Menu key and press ***Yes*** to save the editing session.
6. After you save the editing session, the Module Editor Menu appears. Press the ***PRINT*** soft key in the Module Editor Menu to generate a printout of the selected module.

How to view the steps in a module

1. In the Run Editor Menu, Press the ***'a'... 'j'*** soft key to toggle between lower-case letters (pre-programmed modules) and upper-case letters (user-defined modules.
2. To review the steps in a pre-programmed module, go to the Module Editor Menu and press the ***view*** soft key.

   To review the steps in a user-defined module, press the ***edit*** soft key.

7 Troubleshooting

This section describes most of the problems you could encounter while operating Synergy and how to respond to them. As the operator, you may become aware of a problem when one of the following events occurs:

- a synthesis generates an irregular conductivity trace.
- Flow Test fails.
- a pressure test fails.
- the LCD displays an error message.

Contents

Irregular Conductivity Traces

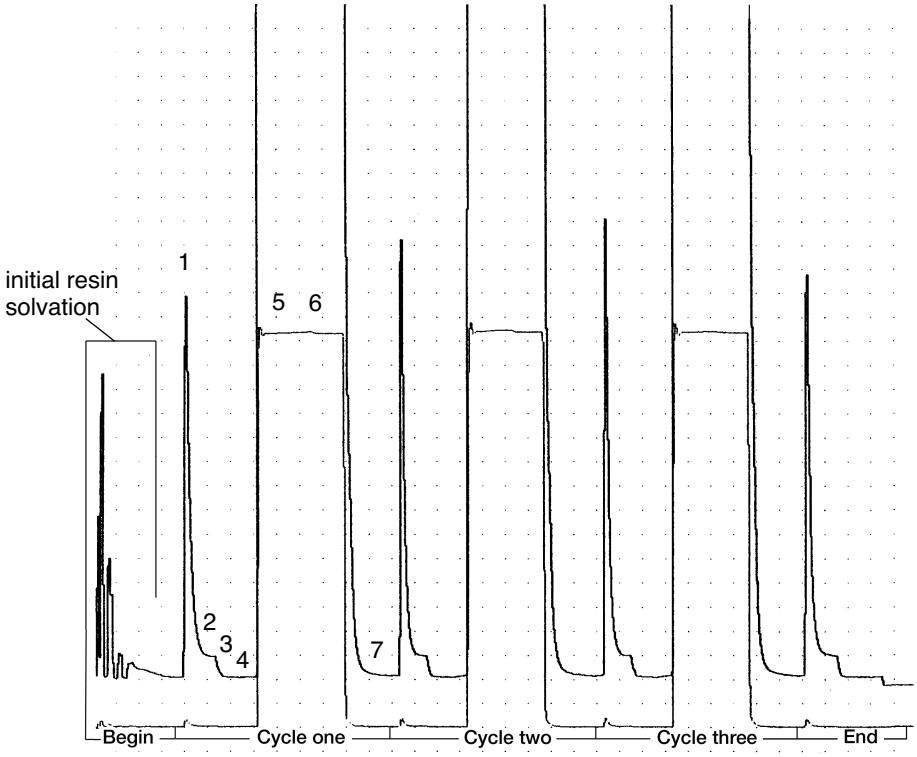


Figure 7-1. *Typical conductivity trace of LAGV synthesis*

The conductivity trace shown in Figure 7-1 has seven characteristic Trace Features that you should see in every synthesis conductivity trace generated by Synergy. See page 2-16 for a description of these seven Trace Features. If all seven features do not appear on a conductivity trace, or if the seven features do not look the same as those in Figure 7-1, it could indicate one of the following:

1. A sequence-dependent, slow deprotection, or incomplete coupling.
2. One or more empty reagent bottles, contaminated or outdated reagents, incorrectly positioned bottles, or a re-used AAC.
3. Malfunctioning instrument hardware.
4. Malfunctioning printer.

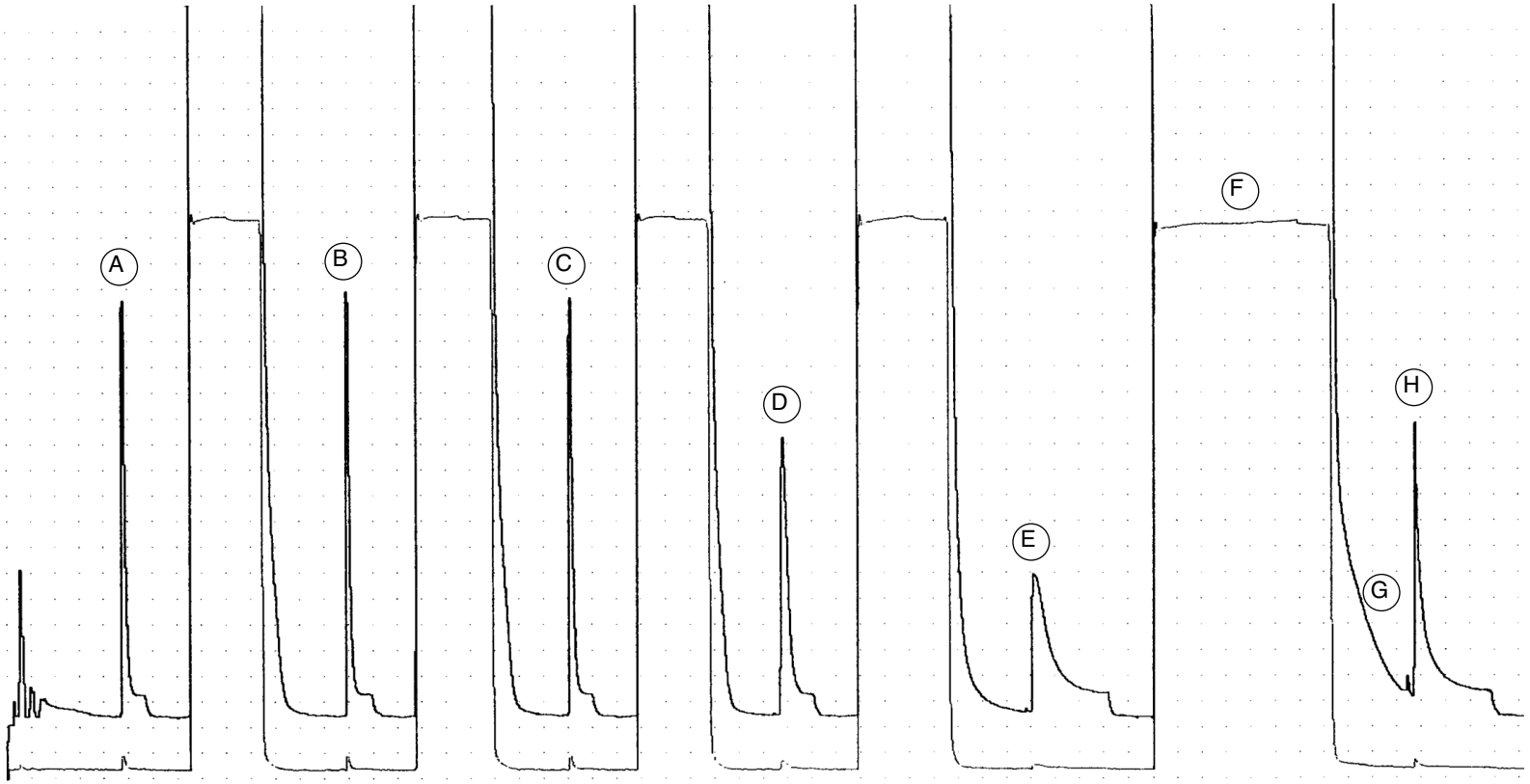


Figure 7-2. *Conductivity trace of a synthesis* (scaled to fit page) *with slow deprotection and incomplete coupling*

The conductivity trace in Figure 7-2 was generated from the synthesis of Ile-Ile-Ile-Ile-Ile-Ile. The first three deprotections (Trace Feature 1) produced three sharp peaks (A, B, C); The fourth deprotection peak (D) is slightly broader and shorter and the fifth peak (E) is considerably broader and shorter. Peaks D and E indicate slower diffusion rates in the peptide-resin (see page 5-5 for an explanation of diffusion rates) and the corresponding coupling times (Trace Features 5 and 6) are automatically increased to compensate (F). However, the sluggish washout of reagents (Trace Feature 7) at G indicates that coupling was incomplete. At G, the conductivity did not return to the baseline in the maximum allotted time, so the following deprotection (H) was abnormally high. This particular coupling may have been improved by double coupling. See page 6-10 for a description of double coupling. See page 2-16 for a discussion of Trace Features.

Sequence-dependent, slow deprotection

Sequence-dependent, slow deprotections arise as a result of the interactions within the peptide-resin and their influence on reagent diffusion rates (see ***The Changing Peptide-Resin Structure*** on page 5-5). Although it is difficult to predict which sequences will reduce reagent diffusion rates, conductivity monitoring can respond to and may at times eliminate their adverse effects on synthesis. Figure 7-2 shows the conductivity trace of a sequence-dependent, slow deprotection.

Reagent bottles and reagent delivery

- Carefully examine all the reagent bottles on Synergy to verify that they all contain sufficient amount of reagent.

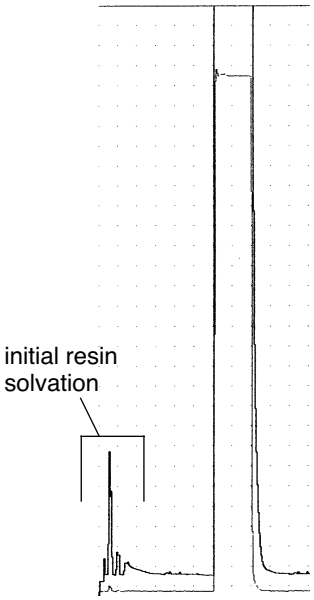


Figure 7-3. *Conductivity trace without deprotection peak, Trace Feature 1*

Figure 7-3 shows a conductivity trace without Trace feature 1, a deprotection peak. (See page 2-16 for a discussion of Trace Features.) This shows what happens when there is no piperidine delivery, due to an empty piperidine bottle on Synergy.

- Verify that reagents have not expired. Applied Biosystems reagents can be safely used up to a year after their date of shipment to your facility. We recommend you record on all Synergy reagent bottles the date of shipment. After preparation (***0.2 M HBTU Reagent Preparation*** on page 2-7), the HBTU reagent (bottle position A) can be used for six weeks.
- Verify that all the reagent bottles have been placed in the correct bottle positions.

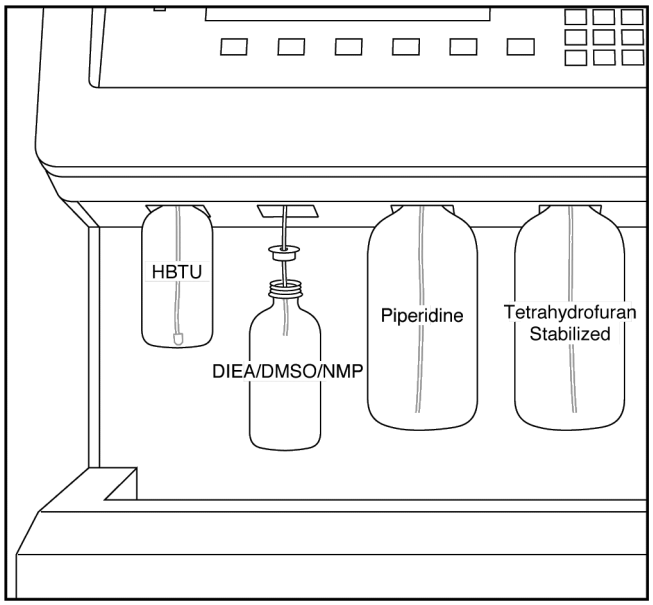


Figure 7-4. *Reagent bottle positions on the front of Synergy*

When all the reagent bottles are in place, from left to right, facing the front of the synthesizer, the labeled reagent bottles should be HBTU [2-(1H-benzotriazol-1-yl)-1,1,3,3 -tetramethyluronium hexafluorophosphate] in position A, DIEA/DMSO/NMP (0.4M N,N-diisopropylethylamine/dimethyl sulfoxide/N-methylpyrrolidone) in position B, Piperidine in position 1, and THF (Tetrahydrofuran) in position 2, DIEA, Piperidine, and THF (Figure 7-4). The DMF (N, N-dimethylformamide) bottle sits on the right side of Synergy in a safety carrier.

If you discover that one or more bottles are not in the correct position

1. Follow the first 6 steps of the ***Synergy Shutdown Procedure*** on page 2-30, but only for those bottle positions that are in the wrong bottle positions.

   If the THF bottle was mistakenly placed in the piperidine bottle position and its contents contaminated with Piperidine, discard the THF.

2. After you complete step 6 of the Synergy Shutdown Procedure, replace the reagents in their correct positions.
3. Prime the reagent lines for the re-positioned bottles.

Re-used AACs

Note ***After a synthesis, the AACs still contain white, inert material, but very little amino acid remains. AACs contain only enough amino acid for a single coupling. They cannot be re-used.***

If you accidentally re-use an AAC in any position, reagent washout (Trace Feature 7) does not return to baseline level for that cycle and all subsequent synthesis cycles, even if the rest of the AACs on the wheel have never been used. (See page 2-16 for a discussion of Trace Features.)

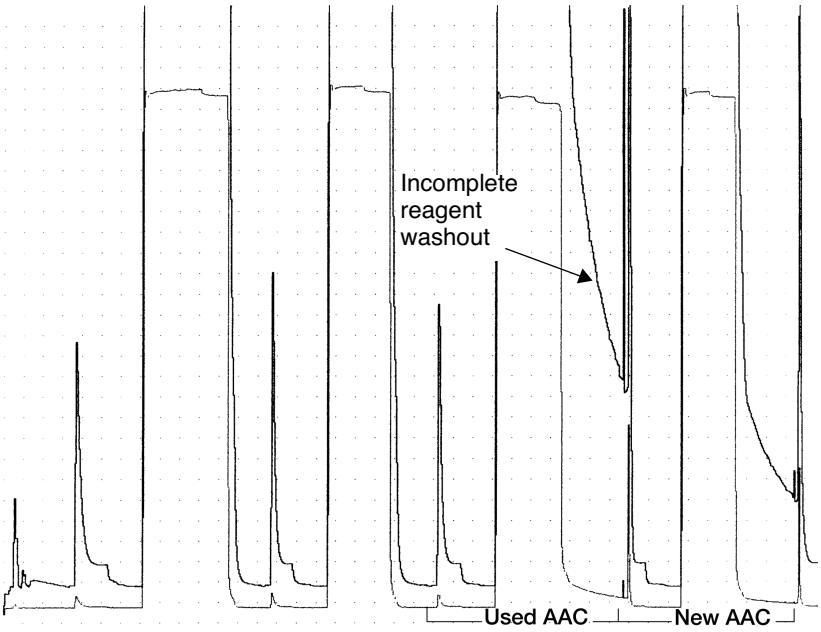


Figure 7-5. *Conductivity trace of synthesis with re-used AAC*

During deprotection, the α-amine on the peptide becomes accessible to a chemical reaction with an activated amino acid. When activators flow through a used AAC, very little, if any activated amino acid is released. The accessible sites on the peptide instead bind to HBTU to form tetramethyl guanidine.[1] In the process, the peptide is partially capped, essentially terminating the synthesis. The incomplete washout results from the presence of basic sites on the resin that prevent rapid washout.

If incomplete reagent washout such as Figure 7-5 demonstrates is present from the beginning of synthesis, it may indicate an excessive delivery of HBTU. Run Flow Test 8 to calibrate HBTU delivery.

1. Gausepohl, H., Pieles, U., and Frank, R.W. 1992. Schiff base analog formation during *in situ* activation by HBTU and TBTU. In *Peptides, Chemistry and Biology, Proceedings of the Twelfth American Peptide Symposium*, ed. J.A. Smith and J. E. Rivier. 523-524. ESCOM, Leiden.

Malfunctioning instrument hardware

Table 7-1. Occurrence of Irregular Trace Features* and Recommended Flow Tests

Irregular/missing feature	Corresponding modules	Run Self Test
Initial resin solvation	b (Begin), f (Flow)	Flow Test 3 (DMF>PSC)
1, 2, or 3	d (Deprotection)	Flow Test 5 (PIP > PSC)
		Flow Test 3 (DM F>PSC)
4	a (Activation)	Leak Test
5	c (Coupling)	Flow Tests 8 (Calibrate HBTU)
		Flow Test 9 (Calibrate DIEA)
6	g (Gurgle)	Flow Test 6 (PUMP/AAC)
7	f (Flow)	Flow Test 3 (DM F > PSC)
End of synthesis	e (End)	Flow Test 4 (THF > PSC)

*See page 2-16 for a discussion of Trace Features.

Table 7-1 provides guidelines for choosing the appropriate Self Test to perform, depending on where irregularities occur in your conductivity trace (see ***Flow Tests*** on page 4-15) and ***Leak Self Test*** on page 4-13). You may be able to pinpoint the source of the irregularity and eliminate it by performing the appropriate corrective actions.

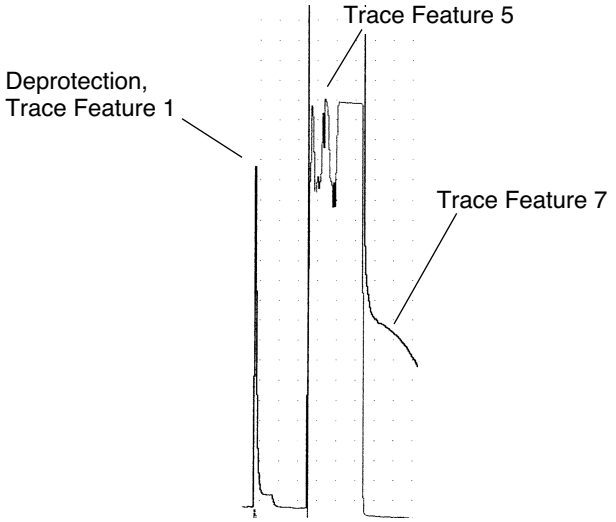


Figure 7-6. *Trace with irregularity at coupling*

Figure 7-6 shows a trace with an irregularity during coupling, due to either a problem with the Synergy "pump" system or with activator delivery. To troubleshoot this irregularity, first perform Flow Test 6 (PUMP/AAC). If this flow test passes, run Flow Tests 8 (Calibrate HBTU) and 9 (Calibrate DIEA).

If Flow Test 6 does not pass, run a Leak Test. If the Leak Test fails at Step 26, check that the left transfer vessel is tightly screwed in place. If the Leak Test continues to fail, call Applied Biosystems Technical Support.

You may also perform a Circuit Test (page 4-36) to scan the electrical circuitry. If you cannot eliminate or determine the cause of your irregular trace, call Applied Biosystems Technical Support.

Printer-related malfunctions

The printer paper may become mis-aligned or jammed in the printer and generate aberrant conductivity traces. If this happens during a synthesis, go to the Setup & Reports Menu and press the ***CANCEL PRINT*** soft key (see ***How to replace or re-align printer paper during synthesis*** on page 2-23).

Check the printer paper and the ink cartridge on the printer. Refer to the *HP Personal Printer Thinkjet Owner's Manual* for Troubleshooting recommendations.

If necessary, you may re-print the conductivity trace after the printer-related problem has been corrected.

How to reprint the conductivity trace

1. In the Main Menu, press the ***setup & report*** soft key.
2. n the Setup & Report Menu, press the ***print reports*** soft key.
3. In the Print Reports Menu, press the ***MONITOR DATA*** soft key.

Pressure Test Failures

A pressure test failure can result from attempting to run Synergy with an empty gas tank, an obstruction in the gas line, or a leak in the pressurized gas system.

Two functions control pressure tests: Function 88 (+PRS TEST), and Function 89 (-PRS TEST). When Function 88 is active, the Synergy controller takes an initial pressure reading and turns off incoming gas for a fixed amount of time. When the fixed amount of time has passed, Function 89 directs the controller to take a second pressure reading and compare it to the initial reading. If the difference between these two readings exceeds the pre-determined acceptable values, the pressure test fails.

Synergy is shipped with a pre-set internal gas pressure range of 4.0 - 6.0 psi. During installation, the internal gas pressure is set to 5.0 ± 0.1 psi.

Empty Gas Tank

For optimal routine operation of Synergy, the high pressure gauge on the external nitrogen tank should not drop below 300 psi, and the low pressure gauge should be set at 60 - 75 psi.

IMPORTANT *Always check the gas tank gauges before starting a synthesis.*

If you attempt to run Synergy with insufficient external gas pressure, the autosampler jaws fail first. When Synergy detects a drop in the internal pressure, the following message appears on the LCD:

During a synthesis, this message could appear only in module b (the beginning of synthesis), when the PSC is tested for leaks, or in module j (the beginning of the pre-programmed cycles), when the AAC is tested for leaks.

If low pressure due to an empty gas tank is detected during module b, you may change the gas tank as soon as Synergy interrupts synthesis, or when the ***PAUSE**** soft key appears in the Run Monitor. See ***How to change the external gas tank*** on page 2-6 for a procedure. When the tank has been replaced, press the ***PAUSE**** soft key to resume synthesis.

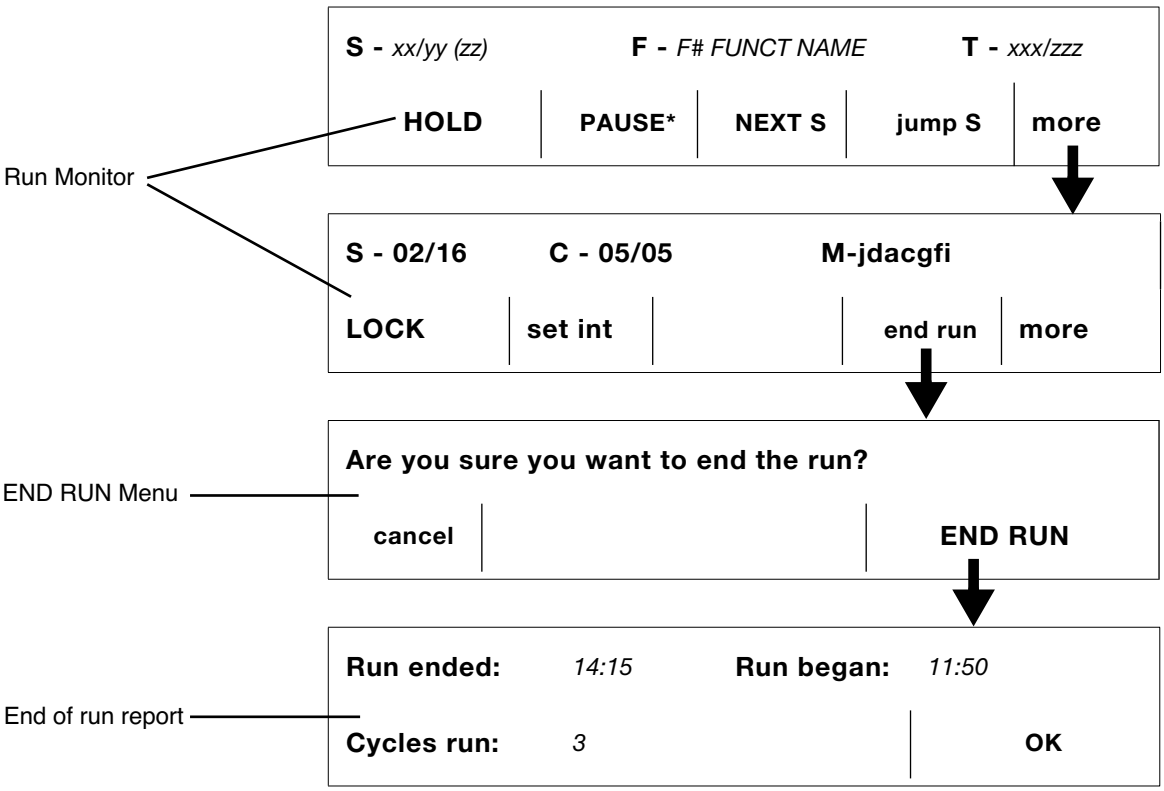
If low pressure due to an empty gas tank is detected during module j, it may be difficult to determine if the synthesis has been affected. Inadequate delivery of reagents may have already compromised activation or coupling in the previous cycle. You

may be able to detect irregularities in the conductivity trace. It might be a waste of reagents, amino acid columns, and time to continue the synthesis.

If you have a conductivity trace of the synthesis before the low pressure was detected and you are not sure whether to continue the synthesis, call Applied Biosystems Technical Support.

How to terminate a synthesis

Follow this procedure to prematurely end the synthesis.



1. In the Run Monitor, press the ***more*** soft key until the ***end run*** soft key appears.
2. Press the ***end run*** soft key. When the message "Are you sure you want to end the run?" appears, press the ***END RUN*** soft key.

   The LCD displays the time the run began and ended.
3. If the jaws are closed when the synthesis ends, go to the Manual Control Menu and activate Function 53, OPEN JAWS. See ***How to manually activate a function*** on page 7-14.

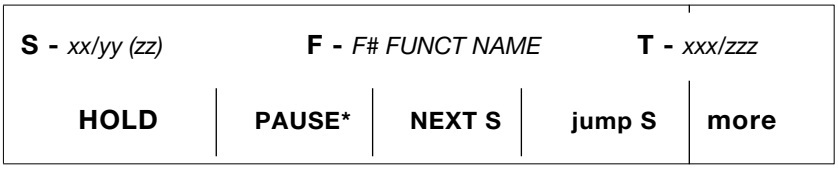
Pressure System Leaks

Synergy cannot operate properly with a leak in the pressure system. It tests the pressure system during module b and module j in a synthesis, during the bottle change procedure, the reagent line prime routines, and some of the Self Tests.

What to do after a pressure test failure

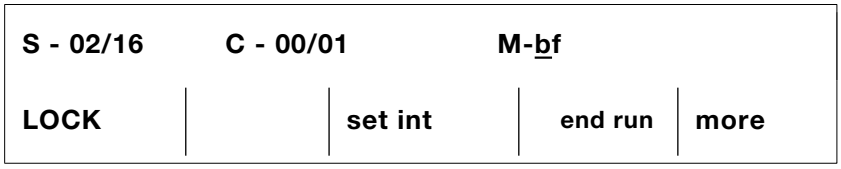
If a leak is detected, the LCD displays a message similar to the one shown here. The value after the word ***diff:*** is the difference between the initial pressure reading and the final reading. Functions 88 and 89 direct the controller to take these two pressure readings.

1. Press the ***OK*** soft key to continue.



The Run Monitor appears with an asterisk (*) after the word ***PAUSE***, which means Synergy has automatically suspended operation. The information in the top line identifies the Step and Function where the pressure failure occurred. When a pressure failure occurs, this line usually displays Function 89 (-PRS TEST).

- If Synergy was running a Self Test when the pressure test failure occurred, note the Step number and refer to Table 7-3 on page 7-16 for a description of the appropriate action.
- If Synergy was running a synthesis when the pressure test failure occurred, press the ***more*** soft key to determine which module was running.



The cursor appears under the module that was running when the pressure test failure occurred. During a synthesis, this would be either module b or module j. Refer to Table 7-2 on page 7-13 for a description of the appropriate corrective action.

2. Perform the appropriate corrective actions.
3. Press the ***more*** soft key until the Run Monitor displays the ***PAUSE**** soft key.
4. Press the ***PAUSE**** soft key to continue the synthesis or the Self Test.

   Whenever a pressure failure occurs, Synergy automatically repeats the pressure test after you press the ***PAUSE**** key to continue operation. If a pressure test repeatedly fails, there could be a leak inside Synergy that only a trained Applied Biosystems Service representative can repair. Call Applied Biosystems Technical Support for assistance.

Pressure Test Failures during a synthesis

Table 7-2. Pressure Test Failures During a Synthesis and Response

When Failure Occurred	Module	Recommended Response
Beginning synthesis	b	Check placement of PSC in luer fittings
		Check Pressure Vent switch
During synthesis	j	Check low pressure gauge
		Check position of AAC in jaws, and action of jaws

Pressure Test Failure at module b, d, or f

- **Check Pressure/Vent switch** Set the Pressure/Vent switch to Pressure during a synthesis. The message ***Pressure out of range*** appears on the LCD when this switch is set to Vent during a synthesis. •
- **Check placement of PSC in luer fittings** Reagent leaks can occur around the peptide synthesis column (PSC) if it has not been pushed firmly into both of its luer fittings. see Figure 4-9 on page 4-13 for an illustration of the PSC placement and make appropriate adjustments.

  If adjusting the PSC placement does not eliminate this pressure failure, examine the rings of the luer fittings on either end of the PSC. These rings should be concentric, without irregularities or cracks. Also check for fluid where the body meets the end of the PSC caps. If you see such leaks, stop the synthesis and discard the PSC.

  If, after replacing the PSC, the pressure test continues to fail, end the synthesis (see ***How to terminate a synthesis*** on page 7-11). When synthesis has ended, run the Leak Self Test (see page 4-13).

Pressure Test Failure at module j

- **Check low pressure gauge** The autosampler jaws fail first when the input gas pressure drops below 60 psi.
- **Check AAC in jaws and action of jaws** To inspect the amino acid column (AAC), first open the autosampler jaws by manually activating Function 53, OPEN JAWS.

How to manually activate a function

1. While the ***PAUSE**** soft key is active in the Run Monitor, press the MAIN key to return to the Main Menu.
2. Press the ***manual control*** soft key to go to the Manual Control Menu.
3. Press the ***function*** soft key to go to the Function Menu (Figure 7-7).
4. Use the numeric keys to enter the number of the function you want to activate, and then press the ***ON*** soft key.

   To open the autosampler jaws, activate Function 53, OPEN JAWS.

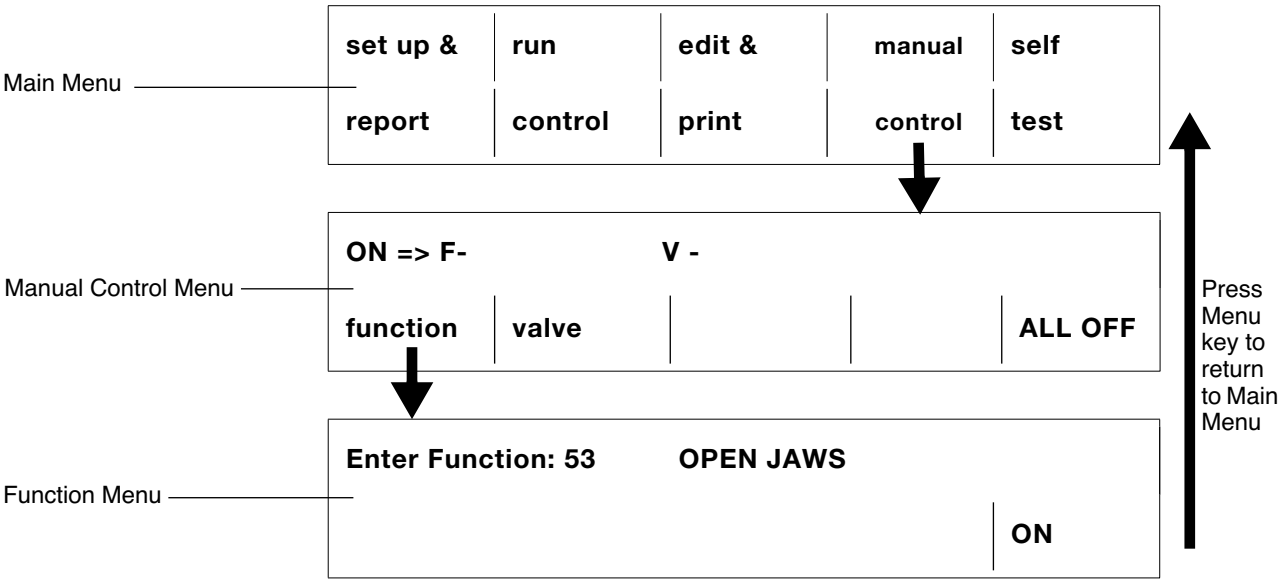


Figure 7-7. *How to manually activate a function*

When the jaws are open, carefully remove the amino acid column wheel, without rotating the wheel hub.

IMPORTANT ***Avoid rotating the wheel hub as you remove and replace the amino acid column wheel during a synthesis interruption.***

Examine both ends of the amino acid column (AAC) for irregularities. The inner rings should be circular and concentric, without irregularities or cracks. If you detect an irregularity, replace the AAC.

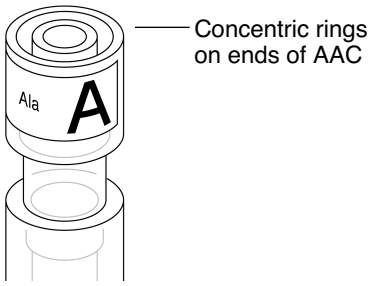


Figure 7-8. *Check the ends of the AAC*

After the AAC has been replaced on the amino acid column wheel, replace the wheel without rotating the wheel hub. Manually activate Function 52, CLOSE JAWS, before continuing the synthesis.

If, after replacing the AAC, the pressure test continues to fail, end the synthesis (see ***How to terminate a synthesis*** on page 7-11). When synthesis has ended, run the Leak Self Test (see page 4-13).

Check that the autosampler jaws close completely on the AAC. If the jaws fail to close completely, call Applied Biosystems Technical Support.

Pressure Test Failures during a Self Test

Use Table 7-3 as a guide to the possible sources of leaks. After you have performed the corrective actions described in this section, Synergy automatically repeats the pressure test that failed. If any pressure test fails repeatedly, call Applied Biosystems Technical Support for assistance.

Pressure Test Failure after a Bottle Change

- **Check bottle seals, bottle cap threads of replaced reagent bottles** Switch the Pressure/Vent switch to Vent and wait 30 seconds for the pressure to drop. Remove any reagent bottles that have recently been replaced and check the bottle seals for cracks or warped surfaces. Replace any defective bottle seals.

WARNING **CHEMICAL HAZARD** ***Reagent bottles are pressurized. With the exception of piperidine, always switch the Pressure Vent switch to "Vent" before unscrewing a reagent bottle***

Piperidine is the only reagent that is on its own pressure system, separate from the other bottles. You may remove the piperidine bottle without venting the pressure system for the other reagents. When you open the piperidine bottle, you hear a small hiss as residual pressure is released.

Table 7-3. Pressure Test Failures During a Self Test and Recommended Response

When Failure Occured	Module	Step where failure occured	Recommended Response
Bottle Change	t	S: 07/07	Check bottle seals, bottle cap threads of replaced reagent bottles
Leak Test	{	S: 02/29	Check HBTU, DIEA, THF bottle seals and caps, DMF bottle lines, transfer vessels
		S: 05/29	Check Piperidine bottle seal and cap
		S: 08/29	Call ABI Service
		S: 11/29	Call ABI Service
		S: 14/29	Call ABI Service
		S: 19/29	Check wheel test fixture in jaws/seals
		S: 23/29	Check PSC and luer fittings
		S: 26/29	Check left transfer vessel
		S: 29/29	Check left and right transfer vessels
Prime THF	u	S: 02/04	Check THF bottle seal and cap
Prime DMF	v	S: 02/06	Check DMF bottle seal and cap
Prime HBTU	w	S: 02/10	Check HBTU bottle seal and cap
Prime DIEA	x	S: 02/10	Check DIEA bottle seal and cap
Prime PIP	y	S: 02/11	Check PIP bottle seal and cap
Flow Test 1	k	S: 04/50	Check flow test calibration tube
Flow Test 2: Calibrate	l	S: 04/22	Check flow test calibration tube
Flow Test 3: DM F-> PSC	m	S: 04/20	Check flow test calibration tube
Flow Test 4: THF-> PSC	n	S: 04/17	Check flow test calibration tube
Flow Test 5: PIP-> PSC	o	S: 04/29	Check flow test calibration tube
Flow Test 6: Pump/AAC	p	S: 06/26	Check test fixture on wheel
Flow Test 7: Pump/Cell	q	S: 04/38	Check flow test calibration tube

Bottles on the front of the instrument have ratchet caps. Use a mirror to check that the threads in the ratchet cap have not been stripped. Replace the reagent bottle in its ratchet cap with the reagent delivery line inside the bottle. Hold the bottle straight up and carefully turn it into the ratchet cap threads. The bottle should fit firmly in place. Do not overtighten the bottle. If the ratchet cap appears to be damaged, call Applied Biosystems Technical Support.

Pressure Test Failure during a Leak Test

- **Check HBTU, DIEA, THF bottle seals and caps, DMF bottle lines.** Follow the preceding instructions for checking bottle seals and ratchet cap threads after a bottle change. If a pressure test fails at Step 2 in the leak test, you do not need to check the piperidine bottle.

  Check that the Pressure line to the DMF bottle is screwed on tightly at the Pressure port fitting. Check that the DMF bottle cap threads are not stripped.

  Check that both transfer vessels are tightly screwed in place.

- **Check piperidine bottle seal and cap.** Follow the instructions for checking bottle seals and caps, described under ***Pressure Test Failure after a Bottle Change*** on page 7-15.
- **Call ABI Service Department.** If a pressure test failure occurs at Step 8, 11, or 14 of the Leak Test, it could indicate loose fittings or valve failures that can only be repaired by trained Applied Biosystems Service person nel. Call Applied Biosystems Service to report these pressure failures.
- **Check wheel test fixture in jaws.** Do not use an AAC or PSC that has been opened to perform this leak test. To examine or replace the column, first press the ***PAUSE**** soft key to continue the Leak Test and open the jaws. After replacing the wheel test fixture, repeat the Leak Self Test from the beginning. Make sure the wheel test fixture is upright in the wheel.

  Check that the autosampler jaws close completely on the wheel test fixture. Insufficient gas pressure can cause jaw failures. If the jaws fail to close completely even with adequate gas pressure, call Applied Biosystems Technical Support.
- **Check PSC and luer fittings.** Reagent leaks can occur around the peptide synthesis column (PSC) if it has not been pushed firmly into both of its luer fittings. Look at Figure 4-10 on page 4-14 for an illustration of the PSC placement and make appropriate adjustments.

  If adjusting the PSC does not eliminate this pressure failure, examine the inner ring of the female luer fitting on either end of the PSC. These rings should be concentric, without irregularities or cracks.
- **Check left and right transfer vessels.** Check that the transfer vessels have been screwed tightly in place, with two delivery lines inside each vessel.

Pressure Test Failure during a reagent prime

- **Check bottle seals and cap.** See instructions on the preceding page for checking bottle seals and cap after a bottle change.

Pressure Test Failure during Flow Test 1, 2, 3, 4, 5, or 7

- **Check PSC**. Reagent leaks can occur around the flow test calibration tube if it has not been pushed firmly into both of its luer fittings. Look at Figure 4-10 on page 4-14 for an illustration of the PSC placement and make appropriate adjustments.

Pressure Test Failure during Flow Test 6: Pump/AAC

- **Check the wheel test fixture in the amino acid column wheel.** Do not use an AAC that has been opened to perform this leak test. Before you can check the wheel test fixture, you have to jump over most of the steps in Flow Test 6 to Step 26: Function 53, OPEN JAWS. See the procedure ***How to jump over steps in a module.***

  Replace the column in position 1 (one) on the amino acid column wheel and repeat Flow Test 6. If the pressure test in Flow Test 6 fails repeatedly, call Applied Biosystems Technical Support for assistance.

How to jump over steps in a module

1. With the ***PAUSE**** soft key active in the Run Monitor, press the ***jump S*** soft key to go to the Jump Step Menu.

   The top line of the LCD in the Jump Step Menu displays the letter name of the module, the Step number in the module, the Function number and name at that step, and the Time, in seconds, allotted to the function. The cursor highlights the Step number (Figure 7-9).

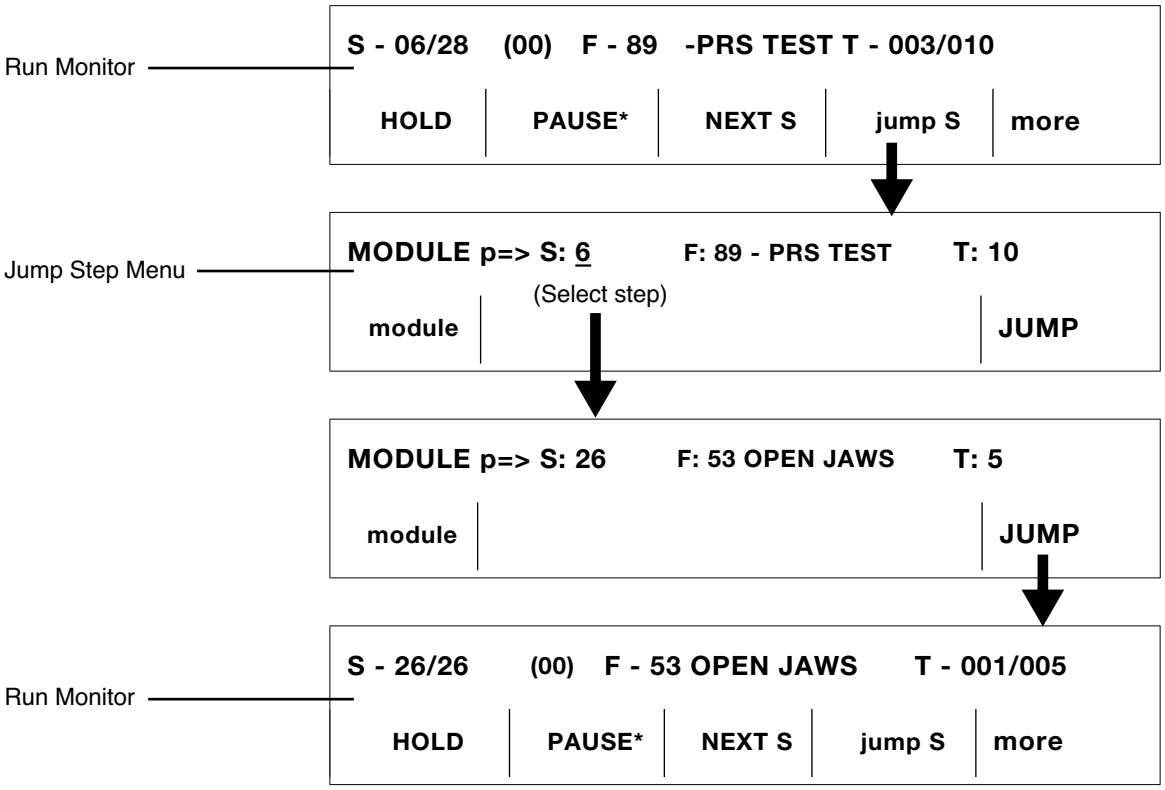


Figure 7-9. *How to Jump Steps in a Module*

2. Use the numeric keys to enter the number of the step the controller should jump to. In Flow Test 6: Pump/AAC (module p), jump to Step 26 to open the jaws after a pressure test failure.
3. Press the ***JUMP*** soft key to return to the Run Monitor.
4. Press the ***PAUSE**** soft key to continue Flow Test 6 at Step 26.

Error Messages

Error messages appear on the LCD as a result of either a mechanical failure or software errors, or after an operator error.

Mechanical Failure or Software Error

Mechanical failures can occur during a synthesis, or during some of the Self Tests. Error messages that describe mechanical failures fall into one of these categories:

- Failure of the autosampler jaws or jaw sensors
- Motor assembly or encoder failure
- Powerfailure

Table 7-4. Error Messages for Mechanical Failures

Mechanical Failure	Error Message
Autosampler jaws or sensors	Open jaw operation failed.
	Close jar operation failed.
	Jaw sensors failed or unplugged.
	Home sensor failed.
Motor assembly or encoder	Motor assembly failed.
	Motor encoder failed.
	Wheel failed to reach desired location.
	The wheel belt is too loose.
Powerfailure	There was a power failure while the synthesizer was running.
	The power was off too long to resume the run.
	A run control error occured before the power fail. The run is paused.
	The run cannot resume because the run file was modified.
	The run cannot resume because the module executing was modified.
	The run cannot resume because the function file was modified.

Autosampler jaw failure

If any of the autosampler error messages listed in Table 7-4 appear on the LCD, first check the gas tank and lower gas regulator gauge. If one of these messages appear even with sufficient gas pressure, call Applied Biosystems Technical Support.

Motor assembly or encoder

If any of the motor assembly or encoder error messages listed in Table 7-4 appear on the LCD, call Applied Biosystems Technical Support.

Powerfailure

There was a power failure while the synthesizer was running. This message appears as soon as power returns but its appearance does not necessarily indicate a problem. Press the ***OK*** soft key to acknowledge the message.

The power was off too long to resume the run. If the powerfailure occurred during a synthesis, Synergy allows a one-hour loss of power during all modules, except module a or j, where it allows only a two-minute outage. This message appears when the length of the powerfailure exceeds these limits. See ***Powerfailures*** on page 2-25 for a discussion of recommended responses to a powerfailure.

A run control error occurred before the powerfailure. The run is paused. If this message appears after a powerfailure, it means that just before the powerfailure, another error message was displayed. Synergy does not "remember" what the error was because the powerfailure erased the error message. Look at the Run Monitor to see what step, function, and module is currently displayed. From this information, you may be able to deduce what occurred just before the powerfailure. If you don't know which error occurred just before the powerfailure, visually inspect the wheel and jaws assembly and check the regulator and internal gas pressure readings before resuming synthesis.

The run file cannot resume because the run file was modified. The run file cannot resume because the module executing was modified. Chapter 6 describes how to use the Run Editor Menu and the Module Editor Menu. You may edit either of these menus during a synthesis without affecting the synthesis, because the Synergy controller looks at the instructions in the Run File only at the beginning of a synthesis. Either of these messages appear after a powerfailure because three sequential events happened: you started a synthesis, you edited a run or a module during the synthesis, and then a powerfailure occurred during the synthesis. When the power returned, the Synergy controller recognized the editions, did not know what instructions to follow, and so ended the synthesis.

The run cannot resume because the function file was modified. When this error message appears, Synergy no longer has values for the measured delivery of HBTU and DIEA. As a result, no activators will be delivered during module a. This message is most likely to occur after an Exception Message has caused an automatic re-start. Run Flow Test 8 and Flow Test 9 to calibrate delivery of HBTU and DIEA before performing a synthesis.

Exception Messages

Software errors—unexpected software events that "confuse" the controller—rarely occur. When one occurs, Synergy emits a loud beep and an "Exception Message" (Table 7-5) appears on the screen for 10 seconds. After 10 seconds, Synergy automatically re-starts itself.

In most instances, you may continue using Synergy after an Exception Message without taking any remedial action. If however, an error message states that "the function file was modified" after an automatic re-start, re-calibrate delivery of

HBTU and DIEA before continuing a synthesis. In these rare instances: end the synthesis that was in progress before the Exception Message appeared, run Flow Tests 8 and 9 and re-start synthesis. Call Applied Biosystems Technical Support for additional assistance.

Table 7-5. Exception Messages

Except 0	TRAPV Instruction
Except 1	Privilege Violation
Bus Error	Trace Interrupt
Address Error	Line 1010 Emulator
Illegal Instruction	Line 1111 Emulator
Divide by Zero	Unexpected Interrupt
CHK Instruction	Spurious Interrupt

Operator-generated Error Messages

Error messages that result from an operator's actions rarely occur during a synthesis that uses the standard, pre-programmed modules. Instead, they follow one of these operator actions:

- Trying to use Manual Control during a synthesis.
- Trying to run a Flow Test during a synthesis.
- Beginning a new synthesis when the printer is printing data.
- Starting Flow Test 6 or the Wheel Test without an AAC in position one.
- Starting a synthesis after an instrument re-set without running Flow Test 8 or Flow Test 9.
- Trying to jump into a software program "loop."
- Incorrectly using Manual Control Menu.
- Incorrectly editing a module or a function.

Table 7-6 lists operator-generated error messages and operator actions that may precede the appearance of the message.

Use Manual Control during a synthesis. The Synergy controller will not let you use the Manual Control Menu while a synthesis is running, unless you first press the ***PAUSE*** soft key in the Run Monitor.

IMPORTANT *When you press the* **PAUSE** *soft key during a synthesis, you interrupt the module that is currently operating. Some module interruptions could interfere with the peptide synthesis and damage the final peptide product.*

Table 7-6. Operator-generated Error Messages

Operator Action	Error Message
Use Manual Control during a synthesis	Pause or end run to operate manually.
Run a Flow Test during a synthesis	Cannot flow test when running.
Begin a synthesis when printer printing data	The monitor file is in use.
Start Flow Test 6 without AAC	Jaw closed with column missing.
Start Wheel Test without AAC	No column in position 1.
Start synthesis without running Flow Test 8 or Flow Test 9	HBTU and DIEA need to be calibrated.
Try to jump into loop	Unmatched Begin Loop/End Loop function.
Incorrect use of Manual Control	Attempted to turn on more than 8 valves. Attempted to operate unavailable valve. May only toggle valve functions. Turn off by using opposite +/- function. Function does not work in Manual Control.
Incorrect edit of module or function	Function cannot execute requested action. Step time too short to execute function. Attempted to turn on more than 8 valves. Attempted to operate unavailable valve. Attempted to execute undefined function. May not move wheel when jaw is closed. May only toggle valve functions. Missing module list or number of repeats. Attempted to run module with no steps. No function number found in module line.

Do not press the ***PAUSE*** soft key without first carefully assessing what module is running and the importance of interrupting the synthesis. As discussed in ***Operator Interruptions to Synthesis*** on page 2-19, only interrupt synthesis at the beginning of either module g, module j, or module i.

Caution ***If liquid waste is backing up into the tubing that goes to external waste ventilation, press the* PAUSE *soft key immediately and empty the waste bottle.***

Run a Flow Test during a synthesis. The Synergy controller will not let you run a flow test simultaneously with a synthesis. If you must run a flow test to check reagent delivery or the conductivity cell, either wait until the synthesis is complete or end the synthesis before the END cycle. To end the synthesis prematurely, see ***How to terminate a synthesis*** on page 7-11.

Begin a synthesis when printer printing data. The buffer, or memory, for the printer can only hold data for one synthesis. As soon as you start a new synthesis, the old data is erased from the buffer. If the printer is printing the old data, the controller cannot erase it to start a new synthesis.

When this error message appears you may let the printer finish printing or you may discontinue printing to start the new synthesis. If you decide to discontinue printing to begin a new synthesis, all the old data will be erased.

How to discontinue data printing

1. Press the ***setup &report*** soft key in the Main Menu.
2. Press the ***print reports*** soft key in the Setup & Report Menu (Figure 7-10).
3. Press the ***CANCEL PRINT*** soft key in the Print Reports Menu.

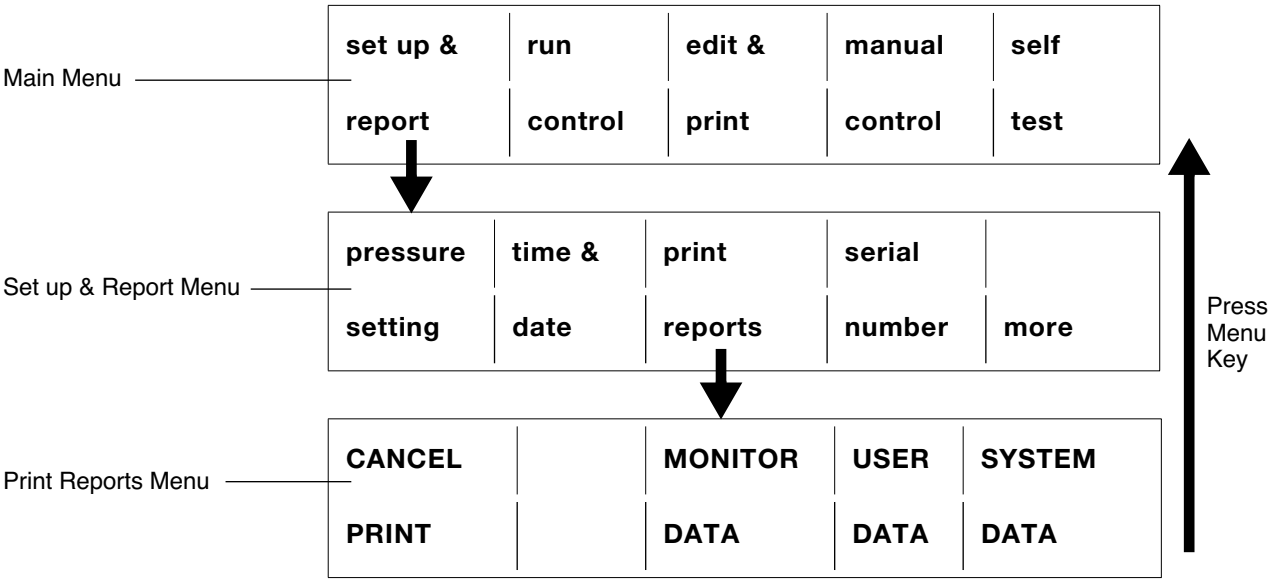


Figure 7-10. *How to go to the Print Reports Menu*

Start Flow Test 6 or the Wheel Self Test without an AAC in position one A wheel test fixture must be in position one of the amino acid column wheel to run either one of these tests. If the jaw sensor does not detect a column in position one during one of these tests, the Synergy controller automatically interrupts the procedure.

To continue the test after this error message

1. Place a wheel test fixture in position one on the wheel.
2. Press the ***OK*** soft key after the error message.
3. Press the ***PAUSE**** soft key in the Run Monitor to continue the test.

Start synthesis without running Flow Test 8 or Flow Test 9. Flow Tests 8 and 9 calibrate the delivery of the activators, HBTU and DIEA. If these flow tests are not run, by default, no activator is delivered during synthesis. When an Exception Message appears on the LCD (see page 7-21), it is automatically followed by a software re-set that may erase the HBTU and DIEA calibration values. If the calibration values are erased, the message ***Function file was modified*** appears on the LCD. You **must** re-calibrate HBTU and DIEA after this message.

Try to jump into a program "loop" A program loop is a set of steps in a module that are repeated until a predefined condition has been satisfied. A loop on Synergy is always bracketed by the function pair Function 3, BEGIN LOOP, and Function 4, END LOOP, or by the function pair Function 5, begin loop, and Function 6, end loop.

These loops are found in a number of modules. During synthesis, for example, one loop implements conductivity monitoring during module d, and another loop repeatedly pumps activation reagents through the amino acid column during activation. There are also loops in Flow Test 1, 5, 7, 8, and 9. Loops also bracket most of the pressure tests, which means that if a pressure test fails, Synergy repeats the pressure test until it passes.

If you follow the procedure ***How to jump over steps in a module*** on page 7-18 and try to jump past the beginning of a loop, the error message "Unmatched Begin Loop/ End Loop function" appears. If you must jump over a Begin Loop, you must also jump over the End Loop function.

Incorrect use of Manual Control or incorrect module edit

With the pre-programmed modules, most Synergy users will seldom, if ever, edit modules or use the Manual Control Menu in such a way that would cause an error message. Advanced users should refer to Chapter 6 for more information on these procedures.

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Numerics

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Appendix I Material Safety Data Sheets and Synergy Waste Profile

The MSDSs found in the following pages are for chemicals that may be purchased from Applied Biosystems. For MSDSs not found in this section, contact the manufacturer or supplier of the related chemicals.

Contents

Material Safety Data Sheets (MSDSs) and Synergy Waste Profile

Acronyms Used in MSDSs

ACGIH	American Conference of Governmental Industrial Hygienists
CAS#	Chemical Abstract Service Reference Number for Specific Pure Chemical
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code Federal Regulations
IDLH	Immediate Danger to Life and Health
LEL	Lower Explosion Limit
NFPA	National Fire Protection Association
NIOSH	National Institute of Occupational Safety and Health
OSHA	Occupational Safety and Health Administration
PEL	Permissable Exposure Limit. The federal OSHA limit, usually expressed as a TWA for an 8-hour work shift.
PPM	Parts Per Million
RTECS	Registry of Toxic Effects of Chemical Substances
SCBA	Self-Contained Breathing Apparatus
TLV	Threshold Limit Value. The ACGIH-recommended TWA, usually for an 8-hour work shift.
TWA	Time Weighted Average
UEL	Upper Explosive Limit

MSDS Table of Contents

MATERIAL SAFETY DATA SHEET

APPLIED BIOSYSTEMS
850 LINCOLN CENTRE DRIVE
FOSTER CITY, CA 94404
(415) 570-6667 (USA)
0925-825650 (UK)

24 HOUR EMERGENCY RESPONSE
NUMBER: 615-366-2000

SUBSTANCE IDENTIFICATION

SUBSTANCE: 0.4 M DIEA/DMSO/NMP REAGENT

TRADE NAMES/SYNONYMS:
ABI MSDS PART # 902035; P/N 401254;
0.4 M N,N-DIISOPROPYLETHYLAMINE/DIMETHYL SULFOXIDE/N-METHYLPYROLLIDONE;

CHEMICAL FAMILY:
MIXTURE

CERCLA RATINGS (SCALE 0-3):	HEALTH=3	FIRE=2	REACTIVITY=0	PERSISTENCE=2
NFPA RATINGS (SCALE 0-4):	HEALTH=2	FIRE=2	REACTIVITY=0	

COMPONENTS AND CONTAMINANTS

COMPONENT: PERCENT: 49.2
DIMETHYL SULFOXIDE
CAS# 67-68-5

COMPONENT: PERCENT: 45.9
1-METHYL-2-PYRROLIDINONE
CAS# 872-50-4

COMPONENT: PERCENT: 4.9
N,N-DIISOPROPYLETHYLAMINE
CAS# 7087-68-5

EXPOSURE LIMITS:
NO OCCUPATIONAL EXPOSURE LIMITS ESTABLISHED BY OSHA, ACGIH, OR NIOSH.

1-METHYL-2-PYRROLIDINONE:
100 PPM (400 MG/M3) DFG MAK TWA
100 PPM BASF CORP. RECOMMENDED TWA

PHYSICAL DATA

DESCRIPTION: LIQUID.

BOILING POINT: NOT AVAILABLE

SPECIFIC GRAVITY: NOT AVAILABLE

VAPOR PRESSURE: NOT AVAILABLE

SOLUBILITY IN WATER: NOT AVAILABLE

FIRE AND EXPLOSION DATA

FIRE AND EXPLOSION HAZARD:
MODERATE FIRE HAZARD WHEN EXPOSED TO HEAT OR FLAME.

VAPOR-AIR MIXTURES ARE EXPLOSIVE ABOVE FLASH POINT.

FLASH POINT: 160 F (71 C) FLAMMABILITY CLASS(OSHA): IIIA

FIREFIGHTING MEDIA:
DRY CHEMICAL, CARBON DIOXIDE, WATER SPRAY OR ALCOHOL-RESISTANT FOAM (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

FOR LARGER FIRES, USE WATER SPRAY, FOG OR ALCOHOL-RESISTANT FOAM (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

FIREFIGHTING:
MOVE CONTAINER FROM FIRE AREA IF YOU CAN DO IT WITHOUT RISK. APPLY COOLING WATER TO SIDES OF CONTAINERS THAT ARE EXPOSED TO FLAMES UNTIL WELL AFTER FIRE IS OUT. STAY AWAY FROM ENDS OF TANKS. FOR MASSIVE FIRE IN CARGO AREA, USE UNMANNED HOSE HOLDER OR MONITOR NOZZLES; IF THIS IS IMPOSSIBLE, WITHDRAW FROM AREA AND LET FIRE BURN. WITHDRAW IMMEDIATELY IN CASE OF RISING SOUND FROM VENTING SAFETY DEVICE OR ANY DISCOLORATION OF TANK DUE TO FIRE. ISOLATE FOR 1/2 MILE IN ALL DIRECTIONS IF TANK, RAIL CAR OR TANK TRUCK IS INVOLVED IN FIRE (1990 EMERGENCY RESPONSE GUIDE-BOOK, DOT P 5800.5, GUIDE PAGE 26).

EXTINGUISH ONLY IF FLOW CAN BE STOPPED; USE WATER IN FLOODING AMOUNTS AS FOG, SOLID STREAMS MAY NOT BE EFFECTIVE. COOL CONTAINERS WITH FLOODING AMOUNTS OF WATER, APPLY FROM AS FAR A DISTANCE AS POSSIBLE. AVOID BREATHING VAPORS, KEEP UPWIND.

TOXICITY

DIMETHYL SULFOXIDE:
IRRITATION DATA: 10 MG/24 HOURS OPEN SKIN-RABBIT MILD; 500 MG/24 HOURS SKIN-RABBIT MILD; 100 MG EYE-RABBIT; 500 MG/24 HOURS EYE-RABBIT MILD.

TOXICITY DATA: 1600 MG/M3/4 HOURS INHALATION-RAT LC50 (38MKAJ); 40 GM/KG SKIN-RAT LD50; 50 GM/KG SKIN-MOUSE LD50; >11 GM/KG SKIN-DOG LD50 (38MKAJ); >11 GM/KG SKIN-MONKEY LD50 (38MKAJ); 14500 MG/KG ORAL-RAT LD50; 7920 MG/KG ORAL-MOUSE LD50; >11 GM/KG ORAL-GUINEA PIG LDLO; >10 GM/KG ORAL-DOG LD50; 12 GM/KG SUBCUTANEOUS-RAT LD50; 14 GM/KG SUBCUTANEOUS-MOUSE LD50; 606 MG/KG INTRAVENOUS-MAN TDLO; 5360 MG/KG INTRAVENOUS-RAT LD50; 3100 MG/KG INTRAVENOUS-MOUSE LD50; 2500 MG/KG INTRAVENOUS-DOG LD50; 200 MG/KG INTRAVENOUS-CAT LDLO; 8200 MG/KG INTRAPERITONEAL-RAT LD50; 2500 MG/KG INTRAPERITONEAL-MOUSE LD50; >5500 MG/KG INTRAPERITONEAL-GUINEA PIG LDLO; 1300 MG/KG UNREPORTED-RAT LD50; MUTAGENIC DATA (RTECS); REPRODUCTIVE EFFECTS DATA (RTECS); TUMORIGENIC DATA (RTECS).
CARCINOGEN STATUS: NONE.
LOCAL EFFECTS: IRRITANT- INHALATION, SKIN, EYE
ACUTE TOXICITY LEVEL: TOXIC BY INHALATION; SLIGHTLY TOXIC BY INGESTION; RELATIVELY NON-TOXIC BY DERMAL ABSORPTION.
TARGET EFFECTS: POISONING MAY AFFECT THE LIVER AND KIDNEYS.
ADDITIONAL DATA: INTERACTIONS WITH MEDICATIONS HAVE BEEN REPORTED.

1-METHYL-2-PYRROLIDINONE (M-PYROL):
IRRIITATION DATA: 100 MG EYE-RABBIT MODERATE.
TOXICITY DATA: 8000 MG/KG SKIN-RABBIT LD50; 3914 MG/KG ORAL-RAT LD50; 4400 MG/KG ORAL-GUINEA PIG LD50; 5130 MG/KG ORAL-MOUSE LD50;

3500 MG/KG ORAL-RABBIT LD50; 80500 UG/KG INTRAVENOUS-RAT LD50; 54500 UG/KG INTRAVENOUS-MOUSE LD50; 63300 UG/KG INTRAVENOUS-DOG LD50; 2472 MG/KG INTRAPERITONEAL-RAT LD50; 3050 MG/KG INTRAPERITONEAL-MOUSE LD50; 7 GM/KG UNREPORTED ROUTE-RAT LD50; MUTAGENIC DATA (RTECS); REPRODUCTIVE EFFECTS DATA (RTECS).
CARCINOGEN STATUS: NONE.
LOCAL EFFECTS: IRRITANT- INHALATION, SKIN, EYES.
ACUTE TOXICITY LEVEL: MODERATELY TOXIC BY INGESTION; SLIGHTLY TOXIC BY DERMAL ABSORPTION.
TARGET EFFECTS: POISONING MAY AFFECT THE CENTRAL NERVOUS SYSTEM.

N,N-DIISOPROPYLETHYLAMINE:
CARCINOGEN STATUS: NONE.
LOCAL EFFECTS: IRRITANT- INHALATION, SKIN, EYE.
ACUTE TOXICITY LEVEL: NO DATA AVAILABLE.
TARGET EFFECTS: NO DATA AVAILABLE.

HEALTH EFFECTS AND FIRST AID

INHALATION:
DIMETHYL SULFOXIDE:
IRRITANT/TOXIC.
ACUTE EXPOSURE- VAPORS MAY CAUSE MODERATE IRRITATION OF THE RESPIRATORY TRACT WITH COUGHING. HIGH CONCENTRATIONS MAY CAUSE SYSTEMIC EFFECTS SUCH AS NAUSEA, VOMITING, CHILLS, CRAMPS, HEADACHE, DIZZINESS, AND LETHARGY. ALLERGIC RESPIRATORY REACTIONS MAY ALSO OCCUR. THE LETHAL DOSE REPORTED IN RATS WAS 1600 MG/M3 FOR 4 HOURS.
CHRONIC EXPOSURE- ANIMALS SHOWED LIVER DAMAGE AND BRONCHOPNEUMONIA ON BEING SUBJECTED TO SPRAY FOR 5 MINUTES, 10 TIMES OVER 15 DAYS, BUT NO EVIDENCE OF TOXICITY ON EXPOSURE TO HEATED VAPOR FOR 30 MINUTES UNDER SIMILAR CONDITIONS. RABBITS EXPOSED TO 25-50 ML/HOUR OF MIST FOR 5 MONTHS DEVELOPED CHEMICAL PNEUMONIA, CLOUDY SWELLING OF THE LIVER, AND SIGNS OF RENAL TOXICITY.

1-METHYL-2-PYRROLIDINONE (M-PYROL):
IRRITANT.
ACUTE EXPOSURE- INHALATION OF VERY HIGH VAPOR CONCENTRATIONS MAY CAUSE MUCOUS MEMBRANE IRRITATION, HEADACHE, GIDDINESS, MENTAL CONFUSION AND NAUSEA. INHALATION OF 180-200 MG/M3 FOR 2 HOURS AND A 6 HOUR EXPOSURE TO SATURATED VAPORS CAUSED NO DEATHS IN MICE AND RATS RESPECTIVELY.
CHRONIC EXPOSURE- PROLONGED EXPOSURE TO VERY HIGH VAPOR CONCENTRATIONS MAY CAUSE HEADACHE, GIDDINESS, MENTAL CONFUSION AND NAUSEA. INHALATION STUDIES IN LABORATORY ANIMALS FAILED TO SHOW ANY GROSS OR HISTOPATHOLOGICAL ABNORMALITIES WHEN EXPOSED TO CONCENTRATIONS OF 50 PPM/8 HOURS/DAY FOR 20 DAYS OR 370 PPM/6 HOURS/DAY FOR 10 DAYS.

N,N-DIISOPROPYLETHYLAMINE:
IRRITANT.
ACUTE EXPOSURE- MAY CAUSE IRRITATION TO THE UPPER RESPIRATORY TRACT, SPASM, INFLAMMATION AND EDEMA OF THE LARYNX AND BRONCHI, CHEMICAL PNEUMONITIS AND PULMONARY EDEMA.
CHRONIC EXPOSURE- NO DATA AVAILABLE.

FIRST AID- REMOVE FROM EXPOSURE AREA TO FRESH AIR IMMEDIATELY. IF BREATHING HAS STOPPED, PERFORM ARTIFICIAL RESPIRATION. KEEP PERSON WARM AND AT REST. TREAT SYMPTOMATICALLY AND SUPPORTIVELY. GET MEDICAL ATTENTION IMMEDIATELY.

SKIN CONTACT:
DIMETHYL SULFOXIDE:
IRRITANT.
ACUTE EXPOSURE- MAY CAUSE IRRITATION WITH ERYTHEMA, ITCHING, SCALING, A TRANSIENT BURNING SENSATION, AND POSSIBLY BLISTERING. IT CAN INITIATE THE IMMEDIATE RELEASE OF HISTAMINE WITH URTICARIAL WHEAL AND FLARE FORMATION. ABSORPTION IS RAPID AND MAY CAUSE A GARLIC-LIKE TASTE AND ODOR TO THE BREATH AND SKIN. LARGE AMOUNTS MAY CAUSE NAUSEA, VOMITING, CRAMPS, DIARRHEA, ANESTHESIA, LETHARGY, DROWSINESS, HEADACHE, CHILLS, CHEST PAINS, BURNING OR ACHING EYES, AND TRANSIENT DISTURBANCES OF COLOR VISION AND PHOTOPHOBIA. TRANSIENT HEMOLYSIS WITH HEMOGLOBINURIA HAS ALSO BEEN REPORTED. ENHANCED IRRITATION, EPIDERMAL VESICULATION, HISTOLOGICAL EVIDENCE OF DERMAL DEATH, AND PERIVASCULAR DERMAL INFILTRATES WERE NOTED AFTER OCCLUDED PATCH TESTING. OCCASIONAL HYPERSENSITIVITY REACTIONS INCLUDING ANAPHYLAXIS HAVE BEEN REPORTED. DUE TO ITS SOLVENT PROPERTIES, DMSO FACILITATES THE ABSORPTION OF SUBSTANCES PRESENT ON THE SKIN WHICH MAY RESULT IN TOXIC EFFECTS
CHRONIC EXPOSURE- 9 MILLILITERS OF 90% DMSO WAS APPLIED TO THE ENTIRE TRUNK OF 20 MEN ONCE DAILY FOR 26 WEEKS. THE EFFECTS NOTED WERE BAD BREATH, TRANSIENT ERYTHEMA, BURNING, AND STINGING. THE DERMATITIS, ACCOMPANIED BY ONLY MODERATE INFLAMMATION REGRESSED AS TREATMENT CONTINUED. DAILY CONTINUOUS APPLICATION WITH OCCLUSION PRODUCED HARDENING OF THE SKIN IN MOST SUBJECTS WITHIN 1 MONTH. CRYSTALLINE LENS ALTERATIONS, RESEMBLING JUVENILE NUCLEAR SCLEROSIS, HAVE BEEN PRODUCED IN SOME ANIMAL SPECIES, BUT NOT IN HUMANS. NO LENS ABNORMALITIES WERE FOUND IN 25 PATIENTS TREATED DAILY WITH UP TO 30 ML APPLIED TOPICALLY FOR 19 MONTHS.

1-METHYL-2-PYRROLIDINONE (M-PYROL):
IRRITANT.
ACUTE EXPOSURE- CONTACT MAY CAUSE MILD IRRITATION.
CHRONIC EXPOSURE- PROLONGED CONTACT HAS BEEN REPORTED TO CAUSE SEVERE DERMATITIS WITH REDNESS, CRACKING, SWELLING, BLISTERS AND EDEMA. REPRODUCTIVE EFFECTS HAVE BEEN REPORTED IN ANIMALS.

N,N-DIISOPROPYLETHYLAMINE:
IRRITANT.
ACUTE EXPOSURE- MAY CAUSE IRRITATION OR DERMATITIS AND POSSIBLY BURNS.
CHRONIC EXPOSURE- NO DATA AVAILABLE.

FIRST AID- REMOVE CONTAMINATED CLOTHING AND SHOES IMMEDIATELY. WASH AFFECTED AREA WITH SOAP OR MILD DETERGENT AND LARGE AMOUNTS OF WATER UNTIL NO EVIDENCE OF CHEMICAL REMAINS (APPROXIMATELY 15-20 MINUTES). GET MEDICAL ATTENTION IMMEDIATELY.

EYE CONTACT:
DIMETHYL SULFOXIDE:
IRRITANT.
ACUTE EXPOSURE- DIRECT CONTACT MAY CAUSE IRRITATION WITH REDNESS, PAIN, AND BLURRED VISION. AQUEOUS SOLUTIONS CONTAINING 75-90% DMSO MAY CAUSE IRRITATION WITH TEMPORARY STINGING AND BURNING. FIFTY PER CENT SOLUTIONS HAVE CAUSED A TRANSIENT BURNING SENSATION. LOWER CONCENTRATIONS HAVE BEEN TOLERATED WELL WITHOUT INJURY TO THE EYE. APPLICATION FULL STRENGTH INTO RABBIT EYES CAUSED PAIN, MODERATE DISCHARGE, CORNEAL EPITHELIUM INJURY, AND DILATION OF CONJUNCTIVAL BLOOD VESSELS BUT NO HEMORRHAGING. THE EYES RETURNED TO NORMAL IN 2 DAYS.
CHRONIC EXPOSURE- REPEATED OR PROLONGED CONTACT WITH IRRITANTS MAY CAUSE CONJUNCTIVITIS.

1-METHYL-2-PYRROLIDINONE (M-PYROL):
IRRITANT.
ACUTE EXPOSURE- EXPOSURE TO VAPORS MAY CAUSE IRRITATION. CONTACT WITH THE LIQUID MAY CAUSE PAINFUL BURNING OR STINGING OF EYES AND LIDS, WATERING OF THE EYES, INFLAMMATION OF CONJUNCTIVA AND TEMPORARY CORNEAL CLOUDING.
CHRONIC EXPOSURE- REPEATED OR PROLONGED EXPOSURE TO IRRITANTS MAY CAUSE CONJUNCTIVITIS.

N,N-DIISOPROPYLETHYLAMINE:
IRRITANT.
ACUTE EXPOSURE- MAY CAUSE SEVERE IRRITATION WITH POSSIBLE BURNS.
CHRONIC EXPOSURE- REPEATED OR PROLONGED CONTACT WITH IRRITANTS MAY CAUSE CONJUNCTIVITIS.

FIRST AID- WASH EYES IMMEDIATELY WITH LARGE AMOUNTS OF WATER OR NORMAL SALINE, OCCASIONALLY LIFTING UPPER AND LOWER LIDS, UNTIL NO EVIDENCE OF CHEMICAL REMAINS (APPROXIMATELY 15-20 MINUTES). GET MEDICAL ATTENTION IMMEDIATELY.

INGESTION:
DIMETHYL SULFOXIDE:
ACUTE EXPOSURE- INGESTION OF LARGE AMOUNTS MAY CAUSE NAUSEA, VOMITING, DIARRHEA, ABDOMINAL PAIN, LETHARGY, AND DROWSINESS.
CHRONIC EXPOSURE- REPEATED LARGE DOSES PRODUCED CRYSTALLINE LENS CHANGES, RESEMBLING JUVENILE NUCLEAR SCLEROSIS, IN SOME ANIMAL SPECIES, BUT NOT IN HUMANS. IN ANIMAL STUDIES, REPEATED DOSES OF 1-5 GM/KG RESULTED IN LIVER NECROSIS AND RENAL LESIONS. REPRODUCTIVE EFFECTS HAVE BEEN REPORTED IN ANIMALS.

1-METHYL-2-PYRROLIDINONE (M-PYROL):
ACUTE EXPOSURE- INGESTION MAY CAUSE GASTROINTESTINAL DISTURBANCES.
CHRONIC EXPOSURE- NINETY DAY FEEDING STUDIES IN LABORATORY ANIMALS AT CONCENTRATIONS UP TO 1% OF THEIR DIET FAILED TO DEMONSTRATE ANY TOXICOLOGICALLY RELEVANT EFFECT. IT HAS BEEN DEMONSTRATED TO BE EMBRYOTOXIC TO RATS AND MICE IN VERY HIGH DOSES.

N,N-DIISOPROPYLETHYLAMINE:
ACUTE EXPOSURE- MAY CAUSE SEVERE IRRITATION OF MOUTH, THROAT AND STOMACH.
CHRONIC EXPOSURE- NO DATA AVAILABLE.

FIRST AID- SEEK MEDICAL ATTENTION IMMEDIATELY. TREAT SYMPTOMATICALLY AND SUPPORTIVELY. MAINTAIN AIRWAY AND RESPIRATION. IF VOMITING OCCURS, KEEP HEAD BELOW HIPS TO PREVENT ASPIRATION. IF A POISONOUS SUBSTANCE HAS BEEN INGESTED, REMOVAL BY EMESIS OR GASTRIC LAVAGE WITH APPROPRIATE PRECAUTIONS TO PREVENT ASPIRATION IS GENERALLY RECOMMENDED PROVIDED THE PATIENT IS CONSCIOUS, NOT CONVULSING AND THERE IS NO SIGN OF CORROSIVE INJURY. ONLY QUALIFIED MEDICAL PERSONNEL SHOULD PERFORM GASTRIC LAVAGE. IF A CORROSIVE SUBSTANCE HAS BEEN INGESTED, DILUTION BY RINSING THE MOUTH AND GIVING WATER OR MILK TO DRINK IS GENERALLY RECOMMENDED. IF PERFORATION HAS OCCURRED OR THE VICTIM IS UNCONSCIOUS, THE VICTIM SHOULD NOT BE GIVEN ANYTHING TO DRINK.

REACTIVITY

REACTIVITY:
STABLE UNDER NORMAL TEMPERATURES AND PRESSURES.

INCOMPATIBILITIES:
DIMETHYL SULFOXIDE:
- ACID ANHYDRIDES: POSSIBLE EXPLOSIVE REACTION.
- ACID HALIDES: POSSIBLE EXPLOSIVE REACTION.
- ACYL HALIDES: VIOLENT OR EXPLOSIVE REACTION.
- ARYL HALIDES: VIOLENT DECOMPOSITION REACTION.
- BORON HYDRIDES: MAY FORM EXPLOSIVE MIXTURE.
- BORON HYDROBORATES: MAY FORM EXPLOSIVE MIXTURE.
- 4(4'-BROMOBENZOYL)ACETANILIDE: MAY EXPLODE AT ELEVATED TEMPERATURES.
- CARBONYL DIISOTHIOCYANATE: EXPLOSIVE REACTION.
- DINITROGEN TETRAOXIDE: VIOLENT OR EXPLOSIVE REACTION.
- IODINE PENTAFLUORIDE: POSSIBLE EXPLOSIVE REACTION.
- METAL NITRATES: FORMS AN EXTREMELY EXPLOSIVE MIXTURE.
- METAL PERCHLORATES: FORMS AN EXTREMELY EXPLOSIVE MIXTURE.
- NITRIC ACID: POSSIBLE EXPLOSION HAZARD.
- OXIDIZERS (STRONG): FIRE AND EXPLOSION HAZARD.
- PERCHLORIC ACID: EXPLODES ON CONTACT.
- PERIODIC ACID: POSSIBLE EXPLOSIVE REACTION.
- PHOSPHOROUS(III) OXIDE: VIOLENT REACTION.
- POTASSIUM: VIOLENT REACTION.
- POTASSIUM PERMANGANATE: IGNITION ON CONTACT.
- SILVER DIFLUORIDE: VIOLENT REACTION.
- SODIUM HYDRIDE: POSSIBLE FIRE AND EXPLOSION AT ELEVATED TEMPERATURES.
- SULFUR TRIOXIDE: EXOTHERMIC REACTION.

1-METHYL-2-PYRROLIDINONE (M-PYROL):
- ACIDS: INCOMPATIBLE.
- OXIDIZERS (STRONG): FIRE AND EXPLOSION HAZARD.

N,N'-DIISOPROPYLETHYLAMINE:
- COATINGS, PLASTICS, FINISHES, RUBBER: MAY BE ATTACKED.
- OXIDIZERS (STRONG): FIRE AND EXPLOSION HAZARD.
- SEE AMINES.

AMINES:
- ACROLEIN: EXOTHERMIC POLYMERIZATION.
- CALCIUM HYPOCHLORITE: FORMATION OF EXPLOSIVE CHLOROAMINE.
- MALEIC ANHYDRIDE: EXPLOSIVE DECOMPOSITION.
- NITROSYL PERCHLORATE: EXPLOSIVE REACTION.
- SODIUM HYPOCHLORITE: FORMATION OF EXPLOSIVE CHLOROAMINE.
- TRI-ISO-BUTYL ALUMINUM: VIOLENT REACTION.

DECOMPOSITION:
THERMAL DECOMPOSITION MAY RELEASE TOXIC AND/OR HAZARDOUS GASES.

POLYMERIZATION:
HAZARDOUS POLYMERIZATION HAS NOT BEEN REPORTED TO OCCUR UNDER NORMAL TEMPERATURES AND PRESSURES.

STORAGE AND DISPOSAL

OBSERVE ALL FEDERAL, STATE AND LOCAL REGULATIONS WHEN STORING OR DISPOSING OF THIS SUBSTANCE. FOR ASSISTANCE, CONTACT THE DISTRICT DIRECTOR OF THE ENVIRONMENTAL PROTECTION AGENCY.

STORAGE

STORE IN ACCORDANCE WITH 29 CFR 1910.106.

STORAGE TEMPERATURE: AMBIENT TO 40 C.
SHELF LIFE: UNKNOWN.

STORE AWAY FROM INCOMPATIBLE SUBSTANCES.

SPILL AND LEAK PROCEDURES

OCCUPATIONAL SPILL:
SHUT OFF IGNITION SOURCES. STOP LEAK IF YOU CAN DO IT WITHOUT RISK. USE WATER SPRAY TO REDUCE VAPORS. FOR SMALL SPILLS, TAKE UP WITH SAND OR OTHER ABSORBENT MATERIAL AND PLACE INTO CONTAINERS FOR LATER DISPOSAL. FOR LARGER SPILLS, DIKE FAR AHEAD OF SPILL FOR LATER DISPOSAL. NO SMOKING, FLAMES OR FLARES IN HAZARD AREA. KEEP UNNECESSARY PEOPLE AWAY; ISOLATE HAZARD AREA AND DENY ENTRY.

PROTECTIVE EQUIPMENT

VENTILATION:
PROVIDE LOCAL EXHAUST OR PROCESS ENCLOSURE VENTILATION TO MEET THE PUBLISHED EXPOSURE LIMITS. VENTILATION EQUIPMENT MUST BE EXPLOSION-PROOF.

RESPIRATOR:
THE FOLLOWING RESPIRATORS ARE RECOMMENDED BASED ON INFORMATION FOUND IN THE PHYSICAL DATA, TOXICITY AND HEALTH EFFECTS SECTIONS. THEY ARE RANKED IN ORDER FROM MINIMUM TO MAXIMUM RESPIRATORY PROTECTION.
THE SPECIFIC RESPIRATOR SELECTED MUST BE BASED ON CONTAMINATION LEVELS FOUND IN THE WORK PLACE, MUST BE BASED ON THE SPECIFIC OPERATION, MUST NOT EXCEED THE WORKING LIMITS OF THE RESPIRATOR AND MUST BE JOINTLY APPROVED BY THE NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH AND THE MINE SAFETY AND HEALTH ADMINISTRATION (NIOSH-MSHA).

ANY TYPE 'C' SUPPLIED-AIR RESPIRATOR WITH A FULL FACEPIECE OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE OR WITH A FULL FACEPIECE, HELMET OR HOOD OPERATED IN CONTINUOUS-FLOW MODE.

ANY SELF-CONTAINED BREATHING APPARATUS WITH A FULL FACEPIECE OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE.

FOR FIREFIGHTING AND OTHER IMMEDIATELY DANGEROUS TO LIFE OR HEALTH CONDITIONS:

ANY SELF-CONTAINED BREATHING APPARATUS THAT HAS A FULL FACEPIECE AND IS OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE.

ANY SUPPLIED-AIR RESPIRATOR THAT HAS A FULL FACEPIECE AND IS OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE IN COMBINATION WITH AN AUXILIARY SELF-CONTAINED BREATHING APPARATUS OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE.

CLOTHING:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE (IMPERVIOUS) CLOTHING AND EQUIPMENT TO PREVENT REPEATED OR PROLONGED SKIN CONTACT WITH THIS SUBSTANCE.

GLOVES:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE GLOVES TO PREVENT CONTACT WITH THIS SUBSTANCE.

EYE PROTECTION:
EMPLOYEE MUST WEAR SPLASH-PROOF OR DUST-RESISTANT SAFETY GOGGLES AND A FACESHIELD TO PREVENT CONTACT WITH THIS SUBSTANCE.

EMERGENCY WASH FACILITIES:
WHERE THERE IS ANY POSSIBILITY THAT AN EMPLOYEE'S EYES AND/OR SKIN MAY BE EXPOSED TO THIS SUBSTANCE, THE EMPLOYER SHOULD PROVIDE AN EYE WASH FOUNTAIN AND QUICK DRENCH SHOWER WITHIN THE IMMEDIATE WORK AREA FOR EMERGENCY USE.

CREATION DATE: 11/08/91 REVISION DATE: July 14, 1993

MATERIAL SAFETY DATA SHEET

APPLIED BIOSYSTEMS
850 LINCOLN CENTRE DRIVE
FOSTER CITY, CA 94404
(415) 570-6667 (USA)
0925-825650 (UK)

24 HOUR EMERGENCY RESPONSE
NUMBER: 615-366-2000

SUBSTANCE IDENTIFICATION

CAS NUMBER: 68-12-2
RTECS NUMBER: LQ2100000

SUBSTANCE: N,N-DIMETHYLFORMAMIDE

TRADE NAMES/SYNONYMS:
ABI MSDS PART # 901822; P/N 400143; DIMETHYLFORMAMIDE; N,N-DIMETHYLMETHANAMIDE; FORMYLDIMETHYLAMINE; DMF (AMIDE); DMFA; DMF; N-FORMYLDIMETHYLAMINE; PEPTIDE SYNTHESIS REAGENT #10; FORMAMIDE, N,N-DIMETHYL-; STCC 4913157; UN 2265; C3H7NO;

CHEMICAL FAMILY:
AMIDE

MOLECULAR FORMULA: H-C-O-N-(C-H3)2

MOLECULAR WEIGHT: 73.09

CERCLA RATINGS(SCALE 0-3): HEALTH=3 FIRE=2 REACTIVITY=0 PERSISTENCE=0
NFPA RATINGS(SCALE 0-4): HEALTH=1 FIRE=2 REACTIVITY=0

COMPONENTS AND CONTAMINANTS

COMPONENT: N,N-DIMETHYLFORMAMIDE PERCENT: 100.0
CAS# 68-12-2

OTHER CONTAMINANTS: NONE

EXPOSURE LIMITS:
N,N-DIMETHYLFORMAMIDE:
10 PPM (30 MG/M3) OSHA TWA (SKIN)
10 PPM (30 MG/M3) ACGIH TWA (SKIN)
10 PPM (30 MG/M3) NIOSH RECOMMENDED TWA (SKIN)
20 PPM (60 MG/M3) DFG MAK TWA (SKIN);
40 PPM (120 MG/M3) DFG MAK 30 MINUTE PEAK, AVERAGE VALUE, 4 TIMES/SHIFT

MEASUREMENT METHOD: SILICA GEL TUBE; METHANOL; GAS CHROMATOGRAPHY WITH FLAME IONIZATION DETECTION; (NIOSH VOL. III # 2004).

PHYSICAL DATA

DESCRIPTION: COLORLESS TO VERY SLIGHTLY YELLOW, HYGROSCOPIC, MOBILE LIQUID WITH A FAINT AMINE-LIKE ODOR. BOILING POINT: 307 F (153 C)

MELTING POINT: -78 F (-61 C) SPECIFIC GRAVITY: 0.9487

VISCOSITY: 0.802 CP @ 25 C VOLATILITY: 100% VAPOR PRESSURE: 3.7 MMHG

EVAPORATION RATE: (N-BUTYL ACETATE=1) <1 PH: 6.7 @ 0.5 M SOLUTION

SOLUBILITY IN WATER: SOLUBLE ODOR THRESHOLD: 100 PPM VAPOR DENSITY: 2.5

SOLVENT SOLUBILITY: SOLUBLE IN ALCOHOL, ACETONE, BENZENE, CARBON TETRACHLORIDE, CHLOROFORM, ETHER, ESTERS, CHLORINATED AND AROMATIC HYDROCARBONS AND OTHER ORGANIC SOLVENTS.

FIRE AND EXPLOSION DATA

FIRE AND EXPLOSION HAZARD:
MODERATE FIRE HAZARD WHEN EXPOSED TO HEAT OR FLAME.

VAPORS ARE HEAVIER THAN AIR AND MAY TRAVEL A CONSIDERABLE DISTANCE TO A SOURCE OF IGNITION AND FLASH BACK.

VAPOR-AIR MIXTURES ARE EXPLOSIVE ABOVE FLASH POINT.

FLASH POINT: 136 F (58 C) (CC) UPPER EXPLOSIVE LIMIT: 15.2% @ 212 F(100 C)

LOWER EXPLOSIVE LIMIT: 2.2% @ 212 F (100 C)

AUTOIGNITION TEMP.: 833 F (445 C) FLAMMABILITY CLASS(OSHA): II

FIREFIGHTING MEDIA:
DRY CHEMICAL, CARBON DIOXIDE, WATER SPRAY OR ALCOHOL-RESISTANT FOAM (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

FOR LARGER FIRES, USE WATER SPRAY, FOG OR ALCOHOL-RESISTANT FOAM (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

ALCOHOL FOAM
(NFPA 325M, FIRE HAZARD PROPERTIES OF FLAMMABLE LIQUIDS, GASES, AND VOLATILE SOLIDS, 1991).

FIREFIGHTING:
MOVE CONTAINER FROM FIRE AREA IF YOU CAN DO IT WITHOUT RISK. APPLY COOLING WATER TO SIDES OF CONTAINERS THAT ARE EXPOSED TO FLAMES UNTIL WELL AFTER FIRE IS OUT. STAY AWAY FROM ENDS OF TANKS. FOR MASSIVE FIRE IN CARGO AREA, USE UNMANNED HOSE HOLDER OR MONITOR NOZZLES; IF THIS IS IMPOSSIBLE, WITHDRAW FROM AREA AND LET FIRE BURN. WITHDRAW IMMEDIATELY IN CASE OF RISING SOUND FROM VENTING SAFETY DEVICE OR ANY DISCOLORATION OF TANK DUE TO FIRE. ISOLATE FOR 1/2 MILE IN ALL DIRECTIONS IF TANK, RAIL CAR OR TANK TRUCK IS INVOLVED IN FIRE (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5, GUIDE PAGE 26).

EXTINGUISH ONLY IF FLOW CAN BE STOPPED; USE WATER IN FLOODING AMOUNTS AS FOG, SOLID STREAMS MAY NOT BE EFFECTIVE. COOL CONTAINERS WITH FLOODING AMOUNTS OF WATER, APPLY FROM AS FAR A DISTANCE AS POSSIBLE. AVOID BREATHING VAPORS, KEEP UPWIND.

TOXICITY

N,N-DIMETHYLFORMAMIDE:
IRRITATION DATA: 100%/24 HOURS SKIN-HUMAN MILD; 10 MG/24 HOURS OPEN SKIN-RABBIT; 20 MG OPEN EYE-RABBIT; 100 MG RINSED EYE-RABBIT SEVERE.
TOXICITY DATA: 9400 MG/M3/2 HOURS INHALATION-MOUSE LC50; 4720 MG/KG SKIN-RABBIT LD50; 2800 MG/KG ORAL-RAT LD50; 3700 MG/KG ORAL-MOUSE LD50; 3800 MG/KG SUBCUTANEOUS-RAT LD50; 4500 MG/KG SUBCUTANEOUS-MOUSE LD50; 2000 MG/KG INTRAVENOUS-RAT LD50; 1800 MG/KG INTRAVENOUS-RABBIT LD50; 2500 MG/KG INTRAVENOUS-MOUSE LD50; 470 MG/KG INTRAVENOUS-DOG LD50; 1050 MG/KG INTRAVENOUS-GUINEA PIG LD50; 1400 MG/KG INTRAPERITONEAL-RAT LD50; 650 MG/KG

INTRAPERITONEAL-MOUSE LD50; 1000 MG/KG INTRAPERITONEAL-RABBIT LD50; 500 MG/KG INTRAPERITONEAL-CAT LD50; 4000 MG/KG INTRAPERITONEAL-GUINEA PIG LDLO; 3900 MG/KG INTRAMUSCULAR-MOUSE LD50; MUTAGENIC DATA (RTECS); REPRODUCTIVE EFFECTS DATA (RTECS).

CARCINOGEN STATUS: HUMAN LIMITED EVIDENCE, ANIMAL INADEQUATE EVIDENCE (IARC GROUP 2B). AN EXCESS RISK FOR TESTICULAR GERM-CELL TUMORS AND CANCER OF THE BUCCAL CAVITY OR PHARYNX AND A NONSIGNIFICANT EXCESS OF LUNG CANCER WERE OBSERVED IN OCCUPATIONALLY EXPOSED PERSONS.

LOCAL EFFECTS: IRRITANT- INHALATION, SKIN, EYES.

ACUTE TOXICITY LEVEL: TOXIC BY INHALATION; MODERATELY TOXIC BY INGESTION; SLIGHTLY TOXIC BY DERMAL ABSORPTION.

TARGET EFFECTS: HEPATOTOXIN. POISONING MAY ALSO AFFECT THE SKIN, KIDNEYS AND CARDIOVASCULAR SYSTEM.

AT INCREASED RISK FROM EXPOSURE: PERSONS WITH SKIN, KIDNEY OR LIVER DISORDERS.

ADDITIONAL DATA: ALCOHOL MAY ENHANCE THE TOXIC EFFECTS.

HEALTH EFFECTS AND FIRST AID

INHALATION:

N,N-DIMETHYLFORMAMIDE:

IRRITANT/HEPATOTOXIN/CARCINOGEN/TOXIC.

3500 PPM IMEDIATELY DANGEROUS TO LIFE OR HEALTH.

ACUTE EXPOSURE- MAY CAUSE IRRITATION TO THE MUCOUS MEMBRANES, FLUSHING OF THE FACE, HEADACHE, DIZZINESS, LOSS OF APPETITE, NAUSEA, VOMITING, COLICKY ABDOMINAL PAIN AND SPASMS, CONSTIPATION, DIARRHEA, HYPERTENSION, HEPATOMEGALY AND OTHER SIGNS OF LIVER INJURY. ANIMALS EXPOSED TO 5000 PPM FOR 6 HOURS SHOWED SIGNS OF LIVER, LUNG AND KIDNEY INJURY. THE LETHAL DOSE REPORTED IN MICE IS 9400 MG/M3/2 HOURS.

CHRONIC EXPOSURE- IN ADDITION TO THE EFFECTS DESCRIBED IN ACUTE INHALATION, EFFECTS FROM OCCUPATIONAL EXPOSURE HAVE INCLUDED FATIGUE, WEAKNESS, NERVOUSNESS, SLEEP DISORDERS, VERTIGO, FACIAL CONGESTION, DIGESTIVE DISTURBANCES, EPIGASTRIC PAIN, CARDIOVASCULAR ABNORMALITIES, NUMBNESS OF THE EXTREMITIES, FUNCTIONAL NERVOUS SYSTEM DISORDERS, AND INCREASED LEVELS OF SERUM AMYLASE SUGGESTING PANCREATITIS. OTHER REPORTED EFFECTS HAVE INCLUDED SLOW COAGULATION TIMES, A SIGNIFICANT REDUCTION IN PLATELET COUNT, INCREASED CHROMOSOMAL ABERRATIONS, AN EXCESS RISK FOR TESTICULAR GERM-CELL TUMORS AND CANCERS OF THE BUCCAL CAVITY OR PHARYNX, AND A NONSIGNIFICANT EXCESS OF LUNG CANCER. PROLONGED EXPOSURE OF DOGS RESULTED IN POLYCYTHEMIA AND CARDIOVASCULAR EFFECTS WITH DECREASED PULSE RATE, LOW BLOOD PRESSURE AND HEART DAMAGE. REPEATED EXPOSURE OF RATS TO CONCENTRATIONS OF 100 TO 450 PPM FOR 4 MONTHS RESULTED IN NECROSIS OF THE LIVER AND MILD KIDNEY DAMAGE. FETAL GROWTH RETARDATION BUT NO MALFORMATION WAS OBSERVED FOLLOWING EXPOSURE OF RATS.

FIRST AID- REMOVE FROM EXPOSURE AREA TO FRESH AIR IMMEDIATELY. IF BREATHING HAS STOPPED, PERFORM ARTIFICIAL RESPIRATION. KEEP PERSON WARM AND AT REST. TREAT SYMPTOMATICALLY AND SUPPORTIVELY. GET MEDICAL ATTENTION IMMEDIATELY.

SKIN CONTACT:

N,N-DIMETHYLFORMAMIDE:

IRRITANT.

ACUTE EXPOSURE- MAY CAUSE IRRITATION, REDNESS, PAIN, AND DESQUAMATION OF THE SKIN. THERE ARE RARE, INCONCLUSIVE REPORTS OF HUMAN SENSITIZATION. EFFECTS OF ITCHING, HYPEREMIA, ABDOMINAL PAIN, VOMITING, AND AN INCREASED IN BLOOD PRESSURE WERE PRODUCED IN ONE CASE OF ACCIDENTIAL EXPOSURE. THIS MATERIAL IS ABSORBED RAPIDLY THROUGH THE SKIN AND OTHER EFFECTS AS DETAILED IN INHALATION MAY OCCUR. FETAL DEATHS HAVE OCCURED WHEN APPLIED

TO THE SKIN OF PREGNANT RATS.
CHRONIC EXPOSURE- PROLONGED OR REPEATED EXPOSURE MAY CAUSE DERMATITIS AS A RESULT OF DEFATTING ACTION. MATERNAL MORTALITY AND AN INCREASED RATE OF EMBRYONIC DEATH WERE OBSERVED IN A STUDY OF PREGNANT RABBITS RECEIVING REPEATED APPLICATIONS.

FIRST AID- REMOVE CONTAMINATED CLOTHING AND SHOES IMMEDIATELY. WASH AFFECTED AREA WITH SOAP OR MILD DETERGENT AND LARGE AMOUNTS OF WATER UNTIL NO EVIDENCE OF CHEMICAL REMAINS (APPROXIMATELY 15-20 MINUTES). GET MEDICAL ATTENTION IMMEDIATELY.

EYE CONTACT:
N,N-DIMETHYLFORMAMIDE:
IRRITANT.
ACUTE EXPOSURE- MAY CAUSE IRRITATION, REDNESS, PAIN, TEARING, AND BLURRED VISION. A 50% SOLUTION IS SLIGHTLY IRRITATING TO RABBIT EYES. A 75%-100% SOLUTION CAUSES A MORE SEVERE REACTION WITH EDEMA OF THE CORNEAL EPITHELIUM. MODERATE CONJUNCTIVITIS HAS ALSO BEEN NOTED.
CHRONIC EXPOSURE- PROLONGED CONTACT WITH VAPORS OR LIQUID MAY CAUSE CONJUNCTIVITIS.

FIRST AID- WASH EYES IMMEDIATELY WITH LARGE AMOUNTS OF WATER OR NORMAL SALINE, OCCASIONALLY LIFTING UPPER AND LOWER LIDS, UNTIL NO EVIDENCE OF CHEMICAL REMAINS (APPROXIMATELY 15-20 MINUTES). GET MEDICAL ATTENTION IMMEDIATELY.

INGESTION:
N,N-DIMETHYLFORMAMIDE:
ACUTE EXPOSURE- MAY CAUSE HEADACHE, DIZZINESS, NAUSEA, VOMITING, DIARRHEA, AND ABDOMINAL PAIN AND SPASMS AND OTHER SYSTEMIC EFFECTS AS DETAILED IN INHALATION. ANIMALS EXPOSED TO A LETHAL DOSE EXHIBITED WEIGHT LOSS, RESTLESSNESS AND IRRITABILITY. PATHOLOGICAL EXAMINATION SHOWED DEPRESSED BONE MARROW ACTIVITY.
CHRONIC EXPOSURE- REPEATED ORAL EXPOSURE IN RATS RESULTED IN LIVER INJURY. THIS SUBSTANCE ADMINISTERED TO PREGNANT RABBITS PRODUCED AN INCREASED INCIDENCE OF FETAL INTERNAL HYDROCEPHALUS; AT MATERNAL TOXIC DOSES, ABORTION, RETARDED FETAL GROWTH, AND ADDITIONAL MALFORMATIONS WERE ALSO OBSERVED.

FIRST AID- IF THE PERSON IS CONSCIOUS AND NOT CONVULSING, INDUCE EMESIS BY GIVING SYRUP OF IPECAC FOLLOWED BY WATER. (IF VOMITING OCCURS KEEP THE HEAD BELOW THE HIPS TO PREVENT ASPIRATION). REPEAT IN 20 MINUTES IF NOT EFFECTIVE INITIALLY. GIVE ACTIVATED CHARCOAL. IN PATIENTS WITH DEPRESSED RESPIRATION OR IF EMESIS IS NOT PRODUCED, PERFORM GASTRIC LAVAGE CAUTIOUSLY (DREISBACH, HANDBOOK OF POISONING, 12TH ED.). TREAT SYMPTOMATICALLY AND SUPPORTIVELY. GASTRIC LAVAGE SHOULD BE PERFORMED BY QUALIFIED MEDICAL PERSONNEL. GET MEDICAL ATTENTION IMMEDIATELY.

ANTIDOTE:
NO SPECIFIC ANTIDOTE. TREAT SYMPTOMATICALLY AND SUPPORTIVELY.

REACTIVITY

REACTIVITY:
STABLE UNDER NORMAL TEMPERATURES AND PRESSURES.

INCOMPATIBILITIES:
N,N-DIMETHYLFORMAMIDE:
ACID CHLORIDES: INCOMPATIBLE.
ALKYL ALUMINUM: VIOLENT REACTION.

2,5-BIS-ENDO-DICHLORO-7-THIABICYCLO(2.2.1)HEPTANE: VIOLENT REACTION.
BOROHYDRIDE: REACTS.
BRASS: MAY BE ATTACKED.
CARBON TETRACHLORIDE: VIOLENT REACTION ABOVE 65 C.
CHLOROFORMATES: INCOMPATIBLE.
COPPER AND ALLOYS: MAY BE ATTACKED.
DIISOCYANATOMETHANE: VIOLENT POLYMERIZATION.
2,5-DIMETHYLPYRROLE + PHOSPHOROUS OXYCHLORIDE: VIOLENT REACTION.
HALOGENS: VIOLENT REACTION.
HALOGENATED HYDROCARBONS + METALS: DANGEROUS REACTION.
IRON: VIOLENT REACTION.
LITHIUM AZIDE: VIOLENT REACTION.
METHYLENE DIISOCYANTE: MAY POLYMERIZE VIOLENTLY.
OXIDIZERS (STRONG): FIRE AND EXPLOSION HAZARD.
PHOSPHOROUS TRIOXIDE: VIOLENT REACTION.
PLASTICS, RUBBER AND COATINGS: MAY BE ATTACKED.
REDUCING AGENTS: INCOMPATIBLE.
SODIUM AND COMPOUNDS: VIOLENT REACTION.
2,4,6-TRICHLORO-1,3,5-TRIAZINE: VIGOROUS REACTION.
TRIETHYLALUMINUM: MAY EXPLODE ON HEATING.

DECOMPOSITION:
THERMAL DECOMPOSITION PRODUCTS MAY INCLUDE TOXIC OXIDES OF CARBON AND NITROGEN.

POLYMERIZATION:
HAZARDOUS POLYMERIZATION HAS NOT BEEN REPORTED TO OCCUR UNDER NORMAL TEMPERATURES AND PRESSURES.

STORAGE AND DISPOSAL

OBSERVE ALL FEDERAL, STATE AND LOCAL REGULATIONS WHEN STORING OR DISPOSING OF THIS SUBSTANCE. FOR ASSISTANCE, CONTACT THE DISTRICT DIRECTOR OF THE ENVIRONMENTAL PROTECTION AGENCY.

STORAGE

STORE IN ACCORDANCE WITH 29 CFR 1910.106.

BONDING AND GROUNDING: SUBSTANCES WITH LOW ELECTROCONDUCTIVITY, WHICH MAY BE IGNITED BY ELECTROSTATIC SPARKS, SHOULD BE STORED IN CONTAINERS WHICH MEET THE BONDING AND GROUNDING GUIDELINES SPECIFIED IN NFPA 77-1983, RECOMMENDED PRACTICE ON STATIC ELECTRICITY.

STORE AWAY FROM INCOMPATIBLE SUBSTANCES.

DISPOSAL

DISPOSAL MUST BE IN ACCORDANCE WITH STANDARDS APPLICABLE TO GENERATORS OF HAZARDOUS WASTE, 40 CFR 262. EPA HAZARDOUS WASTE NUMBER D001.
100 POUND CERCLA SECTION 103 REPORTABLE QUANTITY.

CONDITIONS TO AVOID

AVOID CONTACT WITH HEAT, SPARKS, FLAMES, OR OTHER SOURCES OF IGNITION. VAPORS

MAY BE EXPLOSIVE AND POISONOUS; DO NOT ALLOW UNNECESSARY PERSONNEL IN AREA. DO NOT OVERHEAT CONTAINERS; CONTAINERS MAY VIOLENTLY RUPTURE AND TRAVEL A CONSIDERABLE DISTANCE IN HEAT OF FIRE.

SPILL AND LEAK PROCEDURES

OCCUPATIONAL SPILL:
SHUT OFF IGNITION SOURCES. STOP LEAK IF YOU CAN DO IT WITHOUT RISK. USE WATER SPRAY TO REDUCE VAPORS. FOR SMALL SPILLS, TAKE UP WITH SAND OR OTHER ABSORBENT MATERIAL AND PLACE INTO CONTAINERS FOR LATER DISPOSAL. FOR LARGER SPILLS, DIKE FAR AHEAD OF SPILL FOR LATER DISPOSAL. NO SMOKING, FLAMES OR FLARES IN HAZARD AREA. KEEP UNNECESSARY PEOPLE AWAY; ISOLATE HAZARD AREA AND DENY ENTRY.

PROTECTIVE EQUIPMENT

VENTILATION:
PROCESS ENCLOSURE VENTILATION RECOMMENDED TO MEET PUBLISHED EXPOSURE LIMITS. VENTILATION EQUIPMENT MUST BE EXPLOSION-PROOF.

RESPIRATOR:
THE FOLLOWING RESPIRATORS AND MAXIMUM USE CONCENTRATIONS ARE RECOMMENDATIONS BY THE U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, NIOSH POCKET GUIDE TO CHEMICAL HAZARDS; NIOSH CRITERIA DOCUMENTS OR BY THE U.S. DEPARTMENT OF LABOR, 29 CFR 1910 SUBPART Z.
THE SPECIFIC RESPIRATOR SELECTED MUST BE BASED ON CONTAMINATION LEVELS FOUND IN THE WORK PLACE, MUST NOT EXCEED THE WORKING LIMITS OF THE RESPIRATOR AND BE JOINTLY APPROVED BY THE NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH AND THE MINE SAFETY AND HEALTH ADMINISTRATION (NIOSH-MSHA).

N,N-DIMETHYLFORMAMIDE:
100 PPM- ANY SUPPLIED-AIR RESPIRATOR.
ANY SELF-CONTAINED BREATHING APPARATUS.

250 PPM- ANY SUPPLIED-AIR RESPIRATOR OPERATED IN A CONTINUOUS FLOW MODE.

500 PPM- ANY SELF-CONTAINED BREATHING APPARATUS WITH A FULL FACEPIECE.
ANY SUPPLIED-AIR RESPIRATOR WITH A FULL FACEPIECE.
ANY SUPPLIED-AIR RESPIRATOR THAT HAS A TIGHT-FITTING FACEPIECE AND IS OPERATED IN A CONTINUOUS-FLOW MODE.

3500 PPM- ANY SUPPLIED-AIR RESPIRATOR THAT HAS A FULL FACEPIECE AND IS OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE.

ESCAPE- ANY AIR-PURIFYING, FULL-FACEPIECE RESPIRATOR (GAS MASK) WITH A CHIN-STYLE, FRONT- OR BACK-MOUNTED ORGANIC VAPOR CANISTER.
ANY APPROPRIATE ESCAPE-TYPE, SELF-CONTAINED BREATHING APPARATUS.

FOR FIREFIGHTING AND OTHER IMMEDIATELY DANGEROUS TO LIFE OR HEALTH CONDITIONS:

ANY SELF-CONTAINED BREATHING APPARATUS THAT HAS A FULL FACEPIECE AND IS OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE.

ANY SUPPLIED-AIR RESPIRATOR THAT HAS A FULL FACEPIECE AND IS OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE IN COMBINATION WITH AN AUXILIARY SELF-CONTAINED BREATHING APPARATUS OPERATED IN PRESSURE-DEMAND

OR OTHER POSITIVE-PRESSURE MODE.

CLOTHING:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE (IMPERVIOUS) CLOTHING AND EQUIPMENT TO PREVENT REPEATED OR PROLONGED SKIN CONTACT WITH THIS SUBSTANCE.

GLOVES:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE GLOVES TO PREVENT CONTACT WITH THIS SUBSTANCE.

EYE PROTECTION:
EMPLOYEE MUST WEAR SPLASH-PROOF OR DUST-RESISTANT SAFETY GOGGLES TO PREVENT EYE CONTACT WITH THIS SUBSTANCE.

EMERGENCY EYE WASH: WHERE THERE IS ANY POSSIBILITY THAT AN EMPLOYEE'S EYES MAY BE EXPOSED TO THIS SUBSTANCE, THE EMPLOYER SHOULD PROVIDE AN EYE WASH FOUNTAIN WITHIN THE IMMEDIATE WORK AREA FOR EMERGENCY USE.

CREATION DATE: 07/31/91 REVISION DATE: July 14, 1993

MATERIAL SAFETY DATA SHEET

APPLIED BIOSYSTEMS
850 LINCOLN CENTRE DRIVE
FOSTER CITY, CA 94404
(415) 570-6667 (USA)
0925-825650 (UK)

24 HOUR EMERGENCY RESPONSE
NUMBER: 615-366-2000

SUBSTANCE IDENTIFICATION

CAS NUMBER: 67654-71-1

SUBSTANCE: 2-(1H-BENZOTRIAZOL-1-YL)-1,1,3,3-TETRAMETHYLURONIUM HEXAFLUOROPHOSPHATE

TRADE NAMES/SYNONYMS:
ABI MSDS PART # 901856; P/N 401091; P/N 401278
N,N,N',N'-TETRAMETHYL-O-(BENZOTRIAZOL-1-YL)URONIUM HEXAFLUOROPHOSPHATE;
HBTU; C11H16F6N5OP;

CHEMICAL FAMILY:
TRIAZOLE

DERIVATIVE

MOLECULAR FORMULA: C11-H16-F6-N5-O-P

MOLECULAR WEIGHT: 379.25

CERCLA RATINGS (SCALE 0-3):	HEALTH=U	FIRE=1	REACTIVITY=0	PERSISTENCE=1
NFPA RATINGS (SCALE 0-4):	HEALTH=U	FIRE=1	REACTIVITY=0	

COMPONENTS AND CONTAMINANTS

COMPONENT: 2-(1H-BENZOTRIAZOL-1-YL)-1,1,3,3-TETRAMETHYLURONIUM HEXAFLUOROPHOSPHATE
CAS# 67654-71-1

PERCENT: 100.0

OTHER CONTAMINANTS: NONE

EXPOSURE LIMITS:
NO OCCUPATIONAL EXPOSURE LIMITS ESTABLISHED BY OSHA, ACGIH, OR NIOSH.

PHYSICAL DATA

DESCRIPTION: ODORLESS, WHITE TO OFF-WHITE SOLID.

BOILING POINT: DECOMPOSES

MELTING POINT: 428-464 F (220-240 C) DECOMPOSES

SPECIFIC GRAVITY: NOT AVAILABLE SOLUBILITY IN WATER: SOLUBLE

FIRE AND EXPLOSION DATA

FIRE AND EXPLOSION HAZARD:
SLIGHT FIRE HAZARD WHEN EXPOSED TO HEAT OR FLAME.

DUST-AIR MIXTURES MAY IGNITE OR EXPLODE.

FIREFIGHTING MEDIA:
DRY CHEMICAL, CARBON DIOXIDE, WATER SPRAY OR REGULAR FOAM
(1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

FOR LARGER FIRES, USE WATER SPRAY, FOG OR REGULAR FOAM
(1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

FIREFIGHTING:
MOVE CONTAINER FROM FIRE AREA IF YOU CAN DO IT WITHOUT RISK. DO NOT SCATTER SPILLED MATERIAL WITH HIGH-PRESSURE WATER STREAMS. DIKE FIRE-CONTROL WATER FOR LATER DISPOSAL (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5, GUIDE PAGE 31).

USE AGENTS SUITABLE FOR TYPE OF SURROUNDING FIRE. AVOID BREATHING HAZARDOUS VAPORS, KEEP UPWIND.

TOXICITY

2-(1H-BENZOTRIAZOL-1-YL)-1,1,3,3-TETRAMETHYLURONIUM HEXAFLUOROPHOSPHATE:
CARCINOGEN STATUS: NONE.
ACUTE TOXICITY DATA: NO DATA AVAILABLE.
TARGET EFFECTS: NO DATA AVAILABLE.
AT INCREASED RISK FROM EXPOSURE: PERSONS WITH ASTHMA, BRONCHITIS OR OTHER RESPIRATORY DIFFICULTIES.

HEALTH EFFECTS AND FIRST AID

INHALATION:
2-(1H-BENZOTRIAZOL-1-YL)-1,1,3,3-TETRAMETHYLURONIUM HEXAFLUOROPHOSPHATE:
ACUTE EXPOSURE- MAY CAUSE BRONCHIAL IRRITATION WITH COUGHING AND OTHER SYMPTOMS OF BRONCHIAL DISTRESS.
CHRONIC EXPOSURE- REPEATED OR PROLONGED EXPOSURE MAY CAUSE ALLERGIC RESPIRATORY SYSTEM SENSITIZATION.

FIRST AID- REMOVE FROM EXPOSURE AREA TO FRESH AIR IMMEDIATELY. IF BREATHING HAS STOPPED, PERFORM ARTIFICIAL RESPIRATION. KEEP PERSON WARM AND AT REST. TREAT SYMPTOMATICALLY AND SUPPORTIVELY. GET MEDICAL ATTENTION IMMEDIATELY.

SKIN CONTACT:
2-(1H-BENZOTRIAZOL-1-YL)-1,1,3,3-TETRAMETHYLURONIUM HEXAFLUOROPHOSPHATE:
ACUTE EXPOSURE- MAY CAUSE IRRITATION.
CHRONIC EXPOSURE- REPEATED OR PROLONGED EXPOSURE MAY CAUSE ALLERGIC SENSITIZATION.

FIRST AID- REMOVE CONTAMINATED CLOTHING AND SHOES IMMEDIATELY. WASH AFFECTED AREA WITH SOAP OR MILD DETERGENT AND LARGE AMOUNTS OF WATER UNTIL NO EVIDENCE OF CHEMICAL REMAINS (APPROXIMATELY 15-20 MINUTES). GET MEDICAL ATTENTION IMMEDIATELY.

EYE CONTACT:
2-(1H-BENZOTRIAZOL-1-YL)-1,1,3,3-TETRAMETHYLURONIUM HEXAFLUOROPHOSPHATE:
ACUTE EXPOSURE- MAY CAUSE IRRITATION TO THE EYES AND MUCOUS MEMBRANES OF THE EYELIDS.
CHRONIC EXPOSURE- NO DATA AVAILABLE.

FIRST AID- WASH EYES IMMEDIATELY WITH LARGE AMOUNTS OF WATER OR NORMAL SALINE, OCCASIONALLY LIFTING UPPER AND LOWER LIDS, UNTIL NO EVIDENCE OF CHEMICAL REMAINS (APPROXIMATELY 15-20 MINUTES). GET MEDICAL ATTENTION IMMEDIATELY.

INGESTION:
2-(1H-BENZOTRIAZOL-1-YL)-1,1,3,3-TETRAMETHYLURONIUM HEXAFLUOROPHOSPHATE:
ACUTE EXPOSURE- NO DATA AVAILABLE.
CHRONIC EXPOSURE- REPEATED OR PROLONGED INGESTION OF FLUORINE COMPOUNDS MAY CAUSE FLUOROSIS WITH SYMPTOMS INCLUDING OSTEOSCLEROTIC THICKENING AND CALCIFICATION OF THE LIGAMENTOUS ATTACHMENTS TO THE SKELETON AS WELL AS WEIGHT LOSS, BRITTLENESS OF BONES, REDUCED BONE MARROW SPACE, ANEMIA, WEAKNESS, GENERAL ILL HEALTH, STIFFNESS OF JOINTS, AND DISCOLORATION OF DEVELOPING TEETH. RARELY, CENTRAL NERVOUS SYSTEM INVOLVEMENT MAY OCCUR.

FIRST AID- TREAT SYMPTOMATICALLY AND SUPPORTIVELY. GET MEDICAL ATTENTION IMMEDIATELY. IF VOMITING OCCURS, KEEP HEAD LOWER THAN HIPS TO PREVENT ASPIRATION.

ANTIDOTE:
NO SPECIFIC ANTIDOTE. TREAT SYMPTOMATICALLY AND SUPPORTIVELY.

REACTIVITY

REACTIVITY:
STABLE UNDER NORMAL TEMPERATURES AND PRESSURES.

INCOMPATIBILITIES:
2-(1H-BENZOTRIAZOL-1-YL)-1,1,3,3-TETRAMETHYLURONIUM HEXAFLUOROPHOSPHATE:
ACIDS (STRONG): DECOMPOSES WITH EVOLUTION OF GAS.
OXIDIZERS (STRONG): FIRE AND EXPLOSION HAZARD.

DECOMPOSITION:
THERMAL DECOMPOSITION PRODUCTS MAY INCLUDE TOXIC AND CORROSIVE OXIDES OF PHOSPHOROUS AND HYDROGEN FLUORIDE.

POLYMERIZATION:
HAZARDOUS POLYMERIZATION HAS NOT BEEN REPORTED TO OCCUR UNDER NORMAL TEMPERATURES AND PRESSURES.

STORAGE AND DISPOSAL

OBSERVE ALL FEDERAL, STATE AND LOCAL REGULATIONS WHEN STORING OR DISPOSING OF THIS SUBSTANCE. FOR ASSISTANCE, CONTACT THE DISTRICT DIRECTOR OF THE ENVIRONMENTAL PROTECTION AGENCY.

STORAGE

STORAGE TEMPERATURE: 4 C.
SHELF LIFE: >THREE YEARS.

STORE AWAY FROM INCOMPATIBLE SUBSTANCES.

CONDITIONS TO AVOID

MAY BURN BUT DOES NOT IGNITE READILY. AVOID CONTACT WITH STRONG OXIDIZERS, EXCESSIVE HEAT, SPARKS, OR OPEN FLAME.

SPILL AND LEAK PROCEDURES

OCCUPATIONAL SPILL:
SWEEP UP AND PLACE IN SUITABLE CLEAN, DRY CONTAINERS FOR RECLAMATION OR LATER DISPOSAL. DO NOT FLUSH SPILLED MATERIAL INTO SEWER. KEEP UNNECESSARY PEOPLE AWAY.

PROTECTIVE EQUIPMENT

VENTILATION:
PROVIDE LOCAL EXHAUST VENTILATION. VENTILATION EQUIPMENT MUST BE EXPLOSION PROOF.

RESPIRATOR:
THE FOLLOWING RESPIRATORS ARE RECOMMENDED BASED ON INFORMATION FOUND IN THE PHYSICAL DATA, TOXICITY AND HEALTH EFFECTS SECTIONS. THEY ARE RANKED IN ORDER FROM MINIMUM TO MAXIMUM RESPIRATORY PROTECTION.
THE SPECIFIC RESPIRATOR SELECTED MUST BE BASED ON CONTAMINATION LEVELS FOUND IN THE WORK PLACE, MUST BE BASED ON THE SPECIFIC OPERATION, MUST NOT EXCEED THE WORKING LIMITS OF THE RESPIRATOR AND MUST BE JOINTLY APPROVED BY THE NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH AND THE MINE SAFETY AND HEALTH ADMINISTRATION (NIOSH-MSHA).

ANY DUST AND MIST RESPIRATOR.

ANY AIR-PURIFYING RESPIRATOR WITH A HIGH-EFFICIENCY PARTICULATE FILTER.

ANY POWERED AIR-PURIFYING RESPIRATOR WITH A DUST AND MIST FILTER.

ANY POWERED AIR-PURIFYING RESPIRATOR WITH A HIGH-EFFICIENCY PARTICULATE FILTER.

ANY TYPE 'C' SUPPLIED-AIR RESPIRATOR OPERATED IN THE PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE OR CONTINUOUS-FLOW MODE.

ANY SELF-CONTAINED BREATHING APPARATUS.

FOR FIREFIGHTING AND OTHER IMMEDIATELY DANGEROUS TO LIFE OR HEALTH CONDITIONS:

ANY SELF-CONTAINED BREATHING APPARATUS THAT HAS A FULL FACEPIECE AND IS OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE.

ANY SUPPLIED-AIR RESPIRATOR THAT HAS A FULL FACEPIECE AND IS OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE IN COMBINATION WITH AN AUXILIARY SELF-CONTAINED BREATHING APPARATUS OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE.

CLOTHING:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE (IMPERVIOUS) CLOTHING AND EQUIPMENT TO PREVENT REPEATED OR PROLONGED SKIN CONTACT WITH THIS SUBSTANCE.

GLOVES:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE GLOVES TO PREVENT CONTACT WITH THIS SUBSTANCE.

EYE PROTECTION:
EMPLOYEE MUST WEAR SPLASH-PROOF OR DUST-RESISTANT SAFETY GOGGLES TO PREVENT EYE CONTACT WITH THIS SUBSTANCE.

EMERGENCY EYE WASH: WHERE THERE IS ANY POSSIBILITY THAT AN EMPLOYEE'S EYES MAY BE EXPOSED TO THIS SUBSTANCE, THE EMPLOYER SHOULD PROVIDE AN EYE WASH FOUNTAIN WITHIN THE IMMEDIATE WORK AREA FOR EMERGENCY USE.

CREATION DATE: 09/06/91 REVISION DATE: 02/28/92

MATERIAL SAFETY DATA SHEET

APPLIED BIOSYSTEMS
850 LINCOLN CENTRE DRIVE
FOSTER CITY, CA 94404
(415) 570-6667 (USA)
0925-825650 (UK)

24 HOUR EMERGENCY RESPONSE
NUMBER: 615-366-2000

SUBSTANCE IDENTIFICATION

SUBSTANCE: HOBT (0.2 M)/DMSO/NMP

TRADE NAMES/SYNONYMS:
ABI MSDS PART # 902033; P/N 401279;
1-HYDROXYBENZOTRIAZOLE/DIMETHYL SULFOXIDE/N-METHYLPYROLLIDONE;

CHEMICAL FAMILY:
MIXTURE

CERCLA RATINGS (SCALE 0-3): HEALTH=3 FIRE=2 REACTIVITY=0 PERSISTENCE=2
NFPA RATINGS (SCALE 0-4): HEALTH=2 FIRE=2 REACTIVITY=0

COMPONENTS AND CONTAMINANTS

COMPONENT: PERCENT: 50.22
DIMETHYL SULFOXIDE
CAS# 67-68-5

COMPONENT: PERCENT: 47.12
1-METHYL-2-PYRROLIDINONE
CAS# 872-50-4

COMPONENT: PERCENT: 2.66
1-HYDROXYBENZOTRIAZOLE
CAS# 2592-95-2

EXPOSURE LIMITS:
NO OCCUPATIONAL EXPOSURE LIMITS ESTABLISHED BY OSHA, ACGIH, OR NIOSH.

1-METHYL-2-PYRROLIDINONE:
100 PPM (400 MG/M3) DFG MAK TWA
100 PPM BASF CORP. RECOMMENDED TWA

PHYSICAL DATA

DESCRIPTION: LIQUID.

BOILING POINT: NOT AVAILABLE

SPECIFIC GRAVITY: NOT AVAILABLE

VAPOR PRESSURE: NOT AVAILABLE

SOLUBILITY IN WATER: NOT AVAILABLE

FIRE AND EXPLOSION DATA

FIRE AND EXPLOSION HAZARD:
MODERATE FIRE HAZARD WHEN EXPOSED TO HEAT OR FLAME.

VAPOR-AIR MIXTURES ARE EXPLOSIVE ABOVE FLASH POINT.

FLASH POINT: 190 F (88 C) FLAMMABILITY CLASS(OSHA): IIIA

FIREFIGHTING MEDIA:
DRY CHEMICAL, CARBON DIOXIDE, WATER SPRAY OR ALCOHOL-RESISTANT FOAM (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

FOR LARGER FIRES, USE WATER SPRAY, FOG OR ALCOHOL-RESISTANT FOAM (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

FIREFIGHTING:
MOVE CONTAINER FROM FIRE AREA IF YOU CAN DO IT WITHOUT RISK. APPLY COOLING WATER TO SIDES OF CONTAINERS THAT ARE EXPOSED TO FLAMES UNTIL WELL AFTER FIRE IS OUT. STAY AWAY FROM ENDS OF TANKS. FOR MASSIVE FIRE IN CARGO AREA, USE UNMANNED HOSE HOLDER OR MONITOR NOZZLES; IF THIS IS IMPOSSIBLE, WITHDRAW FROM AREA AND LET FIRE BURN. WITHDRAW IMMEDIATELY IN CASE OF RISING SOUND FROM VENTING SAFETY DEVICE OR ANY DISCOLORATION OF TANK DUE TO FIRE. ISOLATE FOR 1/2 MILE IN ALL DIRECTIONS IF TANK, RAIL CAR OR TANK TRUCK IS INVOLVED IN FIRE (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5, GUIDE PAGE 26).

EXTINGUISH ONLY IF FLOW CAN BE STOPPED; USE WATER IN FLOODING AMOUNTS AS FOG, SOLID STREAMS MAY NOT BE EFFECTIVE. COOL CONTAINERS WITH FLOODING AMOUNTS OF WATER, APPLY FROM AS FAR A DISTANCE AS POSSIBLE. AVOID BREATHING VAPORS, KEEP UPWIND.

TOXICITY

DIMETHYL SULFOXIDE:
IRRITATION DATA: 10 MG/24 HOURS OPEN SKIN-RABBIT MILD; 500 MG/24 HOURS SKIN-RABBIT MILD; 100 MG EYE-RABBIT; 500 MG/24 HOURS EYE-RABBIT MILD.
TOXICITY DATA: 1600 MG/M3/4 HOURS INHALATION-RAT LC50 (38MKAJ); 40 GM/KG SKIN-RAT LD50; 50 GM/KG SKIN-MOUSE LD50; >11 GM/KG SKIN-DOG LD50 (38MKAJ); >11 GM/KG SKIN-MONKEY LD50 (38MKAJ); 14500 MG/KG ORAL-RAT LD50; 7920 MG/KG ORAL-MOUSE LD50; >11 GM/KG ORAL-GUINEA PIG LDLO; >10 GM/KG ORAL-DOG LD50; 12 GM/KG SUBCUTANEOUS-RAT LD50; 14 GM/KG SUBCUTANEOUS-MOUSE LD50; 606 MG/KG INTRAVENOUS-MAN TDLO; 5360 MG/KG INTRAVENOUS-RAT LD50; 3100 MG/KG INTRAVENOUS-MOUSE LD50; 2500 MG/KG INTRAVENOUS-DOG LD50; 200 MG/KG INTRAVENOUS-CAT LDLO; 8200 MG/KG INTRAPERITONEAL-RAT LD50; 2500 MG/KG INTRAPERITONEAL-MOUSE LD50; >5500 MG/KG INTRAPERITONEAL-GUINEA PIG LDLO; 1300 MG/KG UNREPORTED-RAT LD50; MUTAGENIC DATA (RTECS); REPRODUCTIVE EFFECTS DATA (RTECS); TUMORIGENIC DATA (RTECS).
CARCINOGEN STATUS: NONE.
LOCAL EFFECTS: IRRITANT- INHALATION, SKIN, EYE
ACUTE TOXICITY LEVEL: TOXIC BY INHALATION; SLIGHTLY TOXIC BY INGESTION; RELATIVELY NON-TOXIC BY DERMAL ABSORPTION.
TARGET EFFECTS: POISONING MAY AFFECT THE LIVER AND KIDNEYS.
ADDITIONAL DATA: INTERACTIONS WITH MEDICATIONS HAVE BEEN REPORTED.

1-METHYL-2-PYRROLIDINONE (M-PYROL):
IRRTITATION DATA: 100 MG EYE-RABBIT MODERATE.
TOXICITY DATA: 8000 MG/KG SKIN-RABBIT LD50; 3914 MG/KG ORAL-RAT LD50; 4400 MG/KG ORAL-GUINEA PIG LD50; 5130 MG/KG ORAL-MOUSE LD50; 3500 MG/KG ORAL-RABBIT LD50; 80500 UG/KG INTRAVENOUS-RAT LD50;

54500 UG/KG INTRAVENOUS-MOUSE LD50; 63300 UG/KG INTRAVENOUS-DOG LD50; 2472 MG/KG INTRAPERITONEAL-RAT LD50; 3050 MG/KG INTRAPERITONEAL-MOUSE LD50; 7 GM/KG UNREPORTED ROUTE-RAT LD50; MUTAGENIC DATA (RTECS); REPRODUCTIVE EFFECTS DATA (RTECS).
CARCINOGEN STATUS: NONE.
LOCAL EFFECTS: IRRITANT- INHALATION, SKIN, EYES.
ACUTE TOXICITY LEVEL: MODERATELY TOXIC BY INGESTION; SLIGHTLY TOXIC BY DERMAL ABSORPTION.
TARGET EFFECTS: POISONING MAY AFFECT THE CENTRAL NERVOUS SYSTEM.

1-HYDROXYBENZOTRIAZOLE:
CARCINOGEN STATUS: NONE.
ACUTE TOXICITY LEVEL: NO DATA AVAILABLE.
TARGET EFFECTS: NO DATA AVAILABLE.

HEALTH EFFECTS AND FIRST AID

INHALATION:
DIMETHYL SULFOXIDE:
IRRITANT/TOXIC.
ACUTE EXPOSURE- VAPORS MAY CAUSE MODERATE IRRITATION OF THE RESPIRATORY TRACT WITH COUGHING. HIGH CONCENTRATIONS MAY CAUSE SYSTEMIC EFFECTS SUCH AS NAUSEA, VOMITING, CHILLS, CRAMPS, HEADACHE, DIZZINESS, AND LETHARGY. ALLERGIC RESPIRATORY REACTIONS MAY ALSO OCCUR. THE LETHAL DOSE REPORTED IN RATS WAS 1600 MG/M3 FOR 4 HOURS.
CHRONIC EXPOSURE- ANIMALS SHOWED LIVER DAMAGE AND BRONCHOPNEUMONIA ON BEING SUBJECTED TO SPRAY FOR 5 MINUTES, 10 TIMES OVER 15 DAYS, BUT NO EVIDENCE OF TOXICITY ON EXPOSURE TO HEATED VAPOR FOR 30 MINUTES UNDER SIMILAR CONDITIONS. RABBITS EXPOSED TO 25-50 ML/HOUR OF MIST FOR 5 MONTHS DEVELOPED CHEMICAL PNEUMONIA, CLOUDY SWELLING OF THE LIVER, AND SIGNS OF RENAL TOXICITY.

1-METHYL-2-PYRROLIDINONE (M-PYROL):
IRRITANT.
ACUTE EXPOSURE- INHALATION OF VERY HIGH VAPOR CONCENTRATIONS MAY CAUSE MUCOUS MEMBRANE IRRITATION, HEADACHE, GIDDINESS, MENTAL CONFUSION AND NAUSEA. INHALATION OF 180-200 MG/M3 FOR 2 HOURS AND A 6 HOUR EXPOSURE TO SATURATED VAPORS CAUSED NO DEATHS IN MICE AND RATS RESPECTIVELY.
CHRONIC EXPOSURE- PROLONGED EXPOSURE TO VERY HIGH VAPOR CONCENTRATIONS MAY CAUSE HEADACHE, GIDDINESS, MENTAL CONFUSION AND NAUSEA. INHALATION STUDIES IN LABORATORY ANIMALS FAILED TO SHOW ANY GROSS OR HISTOPATHOLOGICAL ABNORMALITIES WHEN EXPOSED TO CONCENTRATIONS OF 50 PPM/8 HOURS/DAY FOR 20 DAYS OR 370 PPM/6 HOURS/DAY FOR 10 DAYS.

1-HYDROXYBENZOTRIAZOLE:
ACUTE EXPOSURE- NO DATA AVAILABLE.
CHRONIC EXPOSURE- NO DATA AVAILABLE.

FIRST AID- REMOVE FROM EXPOSURE AREA TO FRESH AIR IMMEDIATELY. IF BREATHING HAS STOPPED, PERFORM ARTIFICIAL RESPIRATION. KEEP PERSON WARM AND AT REST. TREAT SYMPTOMATICALLY AND SUPPORTIVELY. GET MEDICAL ATTENTION IMMEDIATELY.

SKIN CONTACT:
DIMETHYL SULFOXIDE:
IRRITANT.
ACUTE EXPOSURE- MAY CAUSE IRRITATION WITH ERYTHEMA, ITCHING, SCALING, A TRANSIENT BURNING SENSATION, AND POSSIBLY BLISTERING. IT CAN INITIATE THE IMMEDIATE RELEASE OF HISTAMINE WITH URTICARIAL WHEAL AND FLARE FORMATION. ABSORPTION IS RAPID AND MAY CAUSE A GARLIC-LIKE TASTE AND

ODOR TO THE BREATH AND SKIN. LARGE AMOUNTS MAY CAUSE NAUSEA, VOMITING, CRAMPS, DIARRHEA, ANESTHESIA, LETHARGY, DROWSINESS, HEADACHE, CHILLS, CHEST PAINS, BURNING OR ACHING EYES, AND TRANSIENT DISTURBANCES OF COLOR VISION AND PHOTOPHOBIA. TRANSIENT HEMOLYSIS WITH HEMOGLOBINURIA HAS ALSO BEEN REPORTED. ENHANCED IRRITATION, EPIDERMAL VESICULATION, HISTOLOGICAL EVIDENCE OF DERMAL DEATH, AND PERIVASCULAR DERMAL INFILTRATES WERE NOTED AFTER OCCLUDED PATCH TESTING. OCCASIONAL HYPERSENSITIVITY REACTIONS INCLUDING ANAPHYLAXIS HAVE BEEN REPORTED. DUE TO ITS SOLVENT PROPERTIES, DMSO FACILITATES THE ABSORPTION OF SUBSTANCES PRESENT ON THE SKIN WHICH MAY RESULT IN TOXIC EFFECTS

CHRONIC EXPOSURE- 9 MILLILITERS OF 90% DMSO WAS APPLIED TO THE ENTIRE TRUNK OF 20 MEN ONCE DAILY FOR 26 WEEKS. THE EFFECTS NOTED WERE BAD BREATH, TRANSIENT ERYTHEMA, BURNING, AND STINGING. THE DERMATITIS, ACCOMPANIED BY ONLY MODERATE INFLAMMATION REGRESSED AS TREATMENT CONTINUED. DAILY CONTINUOUS APPLICATION WITH OCCLUSION PRODUCED HARDENING OF THE SKIN IN MOST SUBJECTS WITHIN 1 MONTH. CRYSTALLINE LENS ALTERATIONS, RESEMBLING JUVENILE NUCLEAR SCLEROSIS, HAVE BEEN PRODUCED IN SOME ANIMAL SPECIES, BUT NOT IN HUMANS. NO LENS ABNORMALITIES WERE FOUND IN 25 PATIENTS TREATED DAILY WITH UP TO 30 ML APPLIED TOPICALLY FOR 19 MONTHS.

1-METHYL-2-PYRROLIDINONE (M-PYROL):
IRRITANT.

ACUTE EXPOSURE- CONTACT MAY CAUSE MILD IRRITATION.

CHRONIC EXPOSURE- PROLONGED CONTACT HAS BEEN REPORTED TO CAUSE SEVERE DERMATITIS WITH REDNESS, CRACKING, SWELLING, BLISTERS AND EDEMA. REPRODUCTIVE EFFECTS HAVE BEEN REPORTED IN ANIMALS.

1-HYDROXYBENZOTRIAZOLE:

ACUTE EXPOSURE- NO DATA AVAILABLE.

CHRONIC EXPOSURE- NO DATA AVAILABLE.

FIRST AID- REMOVE CONTAMINATED CLOTHING AND SHOES IMMEDIATELY. WASH AFFECTED AREA WITH SOAP OR MILD DETERGENT AND LARGE AMOUNTS OF WATER UNTIL NO EVIDENCE OF CHEMICAL REMAINS (APPROXIMATELY 15-20 MINUTES). GET MEDICAL ATTENTION IMMEDIATELY.

EYE CONTACT:
DIMETHYL SULFOXIDE:
IRRITANT.

ACUTE EXPOSURE- DIRECT CONTACT MAY CAUSE IRRITATION WITH REDNESS, PAIN, AND BLURRED VISION. AQUEOUS SOLUTIONS CONTAINING 75-90% DMSO MAY CAUSE IRRITATION WITH TEMPORARY STINGING AND BURNING. FIFTY PER CENT SOLUTIONS HAVE CAUSED A TRANSIENT BURNING SENSATION. LOWER CONCENTRATIONS HAVE BEEN TOLERATED WELL WITHOUT INJURY TO THE EYE. APPLICATION FULL STRENGTH INTO RABBIT EYES CAUSED PAIN, MODERATE DISCHARGE, CORNEAL EPITHELIUM INJURY, AND DILATION OF CONJUNCTIVAL BLOOD VESSELS BUT NO HEMORRHAGING. THE EYES RETURNED TO NORMAL IN 2 DAYS.

CHRONIC EXPOSURE- REPEATED OR PROLONGED CONTACT WITH IRRITANTS MAY CAUSE CONJUNCTIVITIS.

1-METHYL-2-PYRROLIDINONE (M-PYROL):
IRRITANT.

ACUTE EXPOSURE- EXPOSURE TO VAPORS MAY CAUSE IRRITATION. CONTACT WITH THE LIQUID MAY CAUSE PAINFUL BURNING OR STINGING OF EYES AND LIDS, WATERING OF THE EYES, INFLAMMATION OF CONJUNCTIVA AND TEMPORARY CORNEAL CLOUDING.

CHRONIC EXPOSURE- REPEATED OR PROLONGED EXPOSURE TO IRRITANTS MAY CAUSE CONJUNCTIVITIS.

1-HYDROXYBENZOTRIAZOLE:
ACUTE EXPOSURE- NO DATA AVAILABLE.
CHRONIC EXPOSURE- NO DATA AVAILABLE.

FIRST AID- WASH EYES IMMEDIATELY WITH LARGE AMOUNTS OF WATER OR NORMAL SALINE, OCCASIONALLY LIFTING UPPER AND LOWER LIDS, UNTIL NO EVIDENCE OF CHEMICAL REMAINS (APPROXIMATELY 15-20 MINUTES). GET MEDICAL ATTENTION IMMEDIATELY.

INGESTION:
DIMETHYL SULFOXIDE:
ACUTE EXPOSURE- INGESTION OF LARGE AMOUNTS MAY CAUSE NAUSEA, VOMITING, DIARRHEA, ABDOMINAL PAIN, LETHARGY, AND DROWSINESS.
CHRONIC EXPOSURE- REPEATED LARGE DOSES PRODUCED CRYSTALLINE LENS CHANGES, RESEMBLING JUVENILE NUCLEAR SCLEROSIS, IN SOME ANIMAL SPECIES, BUT NOT IN HUMANS. IN ANIMAL STUDIES, REPEATED DOSES OF 1-5 GM/KG RESULTED IN LIVER NECROSIS AND RENAL LESIONS. REPRODUCTIVE EFFECTS HAVE BEEN REPORTED IN ANIMALS.

1-METHYL-2-PYRROLIDINONE (M-PYROL):
ACUTE EXPOSURE- INGESTION MAY CAUSE GASTROINTESTINAL DISTURBANCES.
CHRONIC EXPOSURE- NINETY DAY FEEDING STUDIES IN LABORATORY ANIMALS AT CONCENTRATIONS UP TO 1% OF THEIR DIET FAILED TO DEMONSTRATE ANY TOXICOLOGICALLY RELEVANT EFFECT. IT HAS BEEN DEMONSTRATED TO BE EMBRYOTOXIC TO RATS AND MICE IN VERY HIGH DOSES.

1-HYDROXYBENZOTRIAZOLE:
ACUTE EXPOSURE- NO DATA AVAILABLE.
CHRONIC EXPOSURE- NO DATA AVAILABLE.

FIRST AID- SEEK MEDICAL ATTENTION IMMEDIATELY. TREAT SYMPTOMATICALLY AND SUPPORTIVELY. MAINTAIN AIRWAY AND RESPIRATION. IF VOMITING OCCURS, KEEP HEAD BELOW HIPS TO PREVENT ASPIRATION. IF A POISONOUS SUBSTANCE HAS BEEN INGESTED, REMOVAL BY EMESIS OR GASTRIC LAVAGE WITH APPROPRIATE PRECAUTIONS TO PREVENT ASPIRATION IS GENERALLY RECOMMENDED PROVIDED THE PATIENT IS CONSCIOUS, NOT CONVULSING AND THERE IS NO SIGN OF CORROSIVE INJURY. ONLY QUALIFIED MEDICAL PERSONNEL SHOULD PERFORM GASTRIC LAVAGE. IF A CORROSIVE SUBSTANCE HAS BEEN INGESTED, DILUTION BY RINSING THE MOUTH AND GIVING WATER OR MILK TO DRINK IS GENERALLY RECOMMENDED. IF PERFORATION HAS OCCURRED OR THE VICTIM IS UNCONSCIOUS, THE VICTIM SHOULD NOT BE GIVEN ANYTHING TO DRINK.

REACTIVITY

REACTIVITY:
STABLE UNDER NORMAL TEMPERATURES AND PRESSURES.

INCOMPATIBILITIES:
DIMETHYL SULFOXIDE:
ACID ANHYDRIDES: POSSIBLE EXPLOSIVE REACTION.
ACID HALIDES: POSSIBLE EXPLOSIVE REACTION.
ACYL HALIDES: VIOLENT OR EXPLOSIVE REACTION.
ARYL HALIDES: VIOLENT DECOMPOSITION REACTION.
BORON HYDRIDES: MAY FORM EXPLOSIVE MIXTURE.
BORON HYDROBORATES: MAY FORM EXPLOSIVE MIXTURE.
4(4'-BROMOBENZOYL)ACETANILIDE: MAY EXPLODE AT ELEVATED TEMPERATURES.
CARBONYL DIISOTHIOCYANATE: EXPLOSIVE REACTION.
DINITROGEN TETRAOXIDE: VIOLENT OR EXPLOSIVE REACTION.
IODINE PENTAFLUORIDE: POSSIBLE EXPLOSIVE REACTION.
METAL NITRATES: FORMS AN EXTREMELY EXPLOSIVE MIXTURE.
METAL PERCHLORATES: FORMS AN EXTREMELY EXPLOSIVE MIXTURE.

NITRIC ACID: POSSIBLE EXPLOSION HAZARD.
OXIDIZERS (STRONG): FIRE AND EXPLOSION HAZARD.
PERCHLORIC ACID: EXPLODES ON CONTACT.
PERIODIC ACID: POSSIBLE EXPLOSIVE REACTION.
PHOSPHOROUS(III) OXIDE: VIOLENT REACTION.
POTASSIUM: VIOLENT REACTION.
POTASSIUM PERMANGANATE: IGNITION ON CONTACT.
SILVER DIFLUORIDE: VIOLENT REACTION.
SODIUM HYDRIDE: POSSIBLE FIRE AND EXPLOSION AT ELEVATED TEMPERATURES.
SULFUR TRIOXIDE: EXOTHERMIC REACTION.

1-METHYL-2-PYRROLIDINONE (M-PYROL):
ACIDS: INCOMPATIBLE.
OXIDIZERS (STRONG): FIRE AND EXPLOSION HAZARD.

1-HYDROXYBENZOTRIAZOLE:
OXIDIZERS (STRONG): FIRE AND EXPLOSION HAZARD.

DECOMPOSITION:
THERMAL DECOMPOSITION MAY RELEASE TOXIC AND/OR HAZARDOUS GASES.

POLYMERIZATION:
HAZARDOUS POLYMERIZATION HAS NOT BEEN REPORTED TO OCCUR UNDER NORMAL TEMPERATURES AND PRESSURES.

STORAGE AND DISPOSAL

OBSERVE ALL FEDERAL, STATE AND LOCAL REGULATIONS WHEN STORING OR DISPOSING OF THIS SUBSTANCE. FOR ASSISTANCE, CONTACT THE DISTRICT DIRECTOR OF THE ENVIRONMENTAL PROTECTION AGENCY.

STORAGE

STORE IN ACCORDANCE WITH 29 CFR 1910.106.

STORAGE TEMPERATURE: 4 C.
SHELF LIFE: UNKNOWN.

STORE AWAY FROM INCOMPATIBLE SUBSTANCES.

SPILL AND LEAK PROCEDURES

OCCUPATIONAL SPILL:
SHUT OFF IGNITION SOURCES. STOP LEAK IF YOU CAN DO IT WITHOUT RISK. USE WATER SPRAY TO REDUCE VAPORS. FOR SMALL SPILLS, TAKE UP WITH SAND OR OTHER ABSORBENT MATERIAL AND PLACE INTO CONTAINERS FOR LATER DISPOSAL. FOR LARGER SPILLS, DIKE FAR AHEAD OF SPILL FOR LATER DISPOSAL. NO SMOKING, FLAMES OR FLARES IN HAZARD AREA. KEEP UNNECESSARY PEOPLE AWAY; ISOLATE HAZARD AREA AND DENY ENTRY.

PROTECTIVE EQUIPMENT

VENTILATION:
PROVIDE LOCAL EXHAUST OR PROCESS ENCLOSURE VENTILATION TO MEET THE PUBLISHED EXPOSURE LIMITS. VENTILATION EQUIPMENT MUST BE EXPLOSION-PROOF.

RESPIRATOR:
THE FOLLOWING RESPIRATORS ARE RECOMMENDED BASED ON INFORMATION FOUND IN THE PHYSICAL DATA, TOXICITY AND HEALTH EFFECTS SECTIONS. THEY ARE RANKED IN ORDER FROM MINIMUM TO MAXIMUM RESPIRATORY PROTECTION.
THE SPECIFIC RESPIRATOR SELECTED MUST BE BASED ON CONTAMINATION LEVELS FOUND IN THE WORK PLACE, MUST BE BASED ON THE SPECIFIC OPERATION, MUST NOT EXCEED THE WORKING LIMITS OF THE RESPIRATOR AND MUST BE JOINTLY APPROVED BY THE NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH AND THE MINE SAFETY AND HEALTH ADMINISTRATION (NIOSH-MSHA).

ANY TYPE 'C' SUPPLIED-AIR RESPIRATOR WITH A FULL FACEPIECE OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE OR WITH A FULL FACEPIECE, HELMET OR HOOD OPERATED IN CONTINUOUS-FLOW MODE.

ANY SELF-CONTAINED BREATHING APPARATUS WITH A FULL FACEPIECE OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE.

FOR FIREFIGHTING AND OTHER IMMEDIATELY DANGEROUS TO LIFE OR HEALTH CONDITIONS:

ANY SELF-CONTAINED BREATHING APPARATUS THAT HAS A FULL FACEPIECE AND IS OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE.

ANY SUPPLIED-AIR RESPIRATOR THAT HAS A FULL FACEPIECE AND IS OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE IN COMBINATION WITH AN AUXILIARY SELF-CONTAINED BREATHING APPARATUS OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE.

CLOTHING:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE (IMPERVIOUS) CLOTHING AND EQUIPMENT TO PREVENT REPEATED OR PROLONGED SKIN CONTACT WITH THIS SUBSTANCE.

GLOVES:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE GLOVES TO PREVENT CONTACT WITH THIS SUBSTANCE.

EYE PROTECTION:
EMPLOYEE MUST WEAR SPLASH-PROOF OR DUST-RESISTANT SAFETY GOGGLES AND A FACESHIELD TO PREVENT CONTACT WITH THIS SUBSTANCE.

EMERGENCY WASH FACILITIES:
WHERE THERE IS ANY POSSIBILITY THAT AN EMPLOYEE'S EYES AND/OR SKIN MAY BE EXPOSED TO THIS SUBSTANCE, THE EMPLOYER SHOULD PROVIDE AN EYE WASH FOUNTAIN AND QUICK DRENCH SHOWER WITHIN THE IMMEDIATE WORK AREA FOR EMERGENCY USE.

CREATION DATE: 11/11/91 REVISION DATE: 05/29/92

MATERIAL SAFETY DATA SHEET

APPLIED BIOSYSTEMS
850 LINCOLN CENTRE DRIVE
FOSTER CITY, CA 94404
(415) 570-6667 (USA)
0925-825650 (UK)

24 HOUR EMERGENCY RESPONSE
NUMBER: 615-366-2000

SUBSTANCE IDENTIFICATION

CAS NUMBER: 110-89-4
RTECS NUMBER: TM3500000

SUBSTANCE: PIPERIDINE

TRADE NAMES/SYNONYMS:
ABI MSDS PART # 901837; P/N 400629; HEXAZANE; CYCLOPENTIMINE; CYPENTIL; AZACYCLOHEXANE; PERHYDROPYRIDINE; PENTAMETHYLENEIMINE; HEXAHYDROPYRIDINE; PEPTIDE SYNTHESIS REAGENT # 1; UN 2401; C5H11N;

CHEMICAL FAMILY:
PIPERIDINE

MOLECULAR FORMULA:(C-H2)5-N-H MOL WT: 85.15

CERCLA RATINGS(SCALE 0-3): HEALTH=3 FIRE=3 REACTIVITY=3 PERSISTENCE=0
NFPA RATINGS(SCALE 0-4): HEALTH=2 FIRE=3 REACTIVITY=3

COMPONENTS AND CONTAMINANTS

COMPONENT: PIPERIDINE PERCENT: 100
CAS# 110-89-4

OTHER CONTAMINANTS: NONE.

EXPOSURE LIMITS:
NO OCCUPATIONAL EXPOSURE LIMITS ESTABLISHED BY OSHA, ACGIH, OR NIOSH.

PIPERIDINE:
1 PPM AIHA RECOMMENDED TWA

1000 POUNDS SARA SECTION 302 THRESHOLD PLANNING QUANTITY
1 POUND SARA SECTION 304 REPORTABLE QUANTITY

PHYSICAL DATA

DESCRIPTION: CLEAR, COLORLESS LIQUID WITH A PEPPER-LIKE OR FISHY ODOR AND A SOAPY FEEL. BOILING POINT: 223 F (106 C)

MELTING POINT: 16 TO 19 F (-9 TO -7 C) SPECIFIC GRAVITY: 0.862

VOLATILITY: 100% VAPOR PRESSURE: 40 MMHG @ 29 C PH: STRONG BASE

SOLUBILITY IN WATER: 100% VAPOR DENSITY: 3.0

SOLVENT SOLUBILITY: SOLUBLE IN ACETONE, ALCOHOL, BENZENE, CHLOROFORM AND

ETHER.

PKA: 11.123 @ 25 C

REFRACTIVE INDEX: 1.4534 @ 20 C

CONVERSION FACTOR: 1 PPM=3.48 MG/M3

FIRE AND EXPLOSION DATA

FIRE AND EXPLOSION HAZARD:
DANGEROUS FIRE HAZARD WHEN EXPOSED TO HEAT OR FLAME.

MODERATE EXPLOSION HAZARD WHEN EXPOSED TO HEAT OR FLAME.

VAPOR-AIR MIXTURES ARE EXPLOSIVE.

VAPORS ARE HEAVIER THAN AIR AND MAY TRAVEL A CONSIDERABLE DISTANCE TO A SOURCE OF IGNITION AND FLASH BACK.

DUE TO LOW ELECTROCONDUCTIVITY OF THE SUBSTANCE, FLOW OR AGITATION MAY GENERATE ELECTROSTATIC CHARGES RESULTING IN SPARKS WITH POSSIBLE IGNITION.

FLASH POINT: 61 F (16 C) (CC) FLAMMABILITY CLASS(OSHA): IB

FIREFIGHTING MEDIA:
DRY CHEMICAL, CARBON DIOXIDE, WATER SPRAY OR REGULAR FOAM (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

FOR LARGER FIRES, USE WATER SPRAY, FOG OR REGULAR FOAM (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

ALCOHOL FOAM
(NFPA 325M, FIRE HAZARD PROPERTIES OF FLAMMABLE LIQUIDS, GASES, AND VOLATILE SOLIDS, 1991).

FIREFIGHTING:
MOVE CONTAINER FROM FIRE AREA IF YOU CAN DO IT WITHOUT RISK. DO NOT GET WATER INSIDE CONTAINER. APPLY COOLING WATER TO SIDES OF CONTAINERS THAT ARE EXPOSED TO FLAMES UNTIL WELL AFTER FIRE IS OUT. STAY AWAY FROM ENDS OF TANKS. WITHDRAW IMMEDIATELY IN CASE OF RISING SOUND FROM VENTING SAFETY DEVICE OR ANY DISCOLORATION OF TANK DUE TO FIRE. ISOLATE FOR 1/2 MILE IN ALL DIRECTIONS IF TANK, RAIL CAR OR TANK TRUCK IS INVOLVED IN FIRE (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800,5, GUIDE PAGE 29).

EXTINGUISH ONLY IF FLOW CAN BE STOPPED. COOL CONTAINERS WITH FLOODING AMOUNTS OF WATER FROM AS FAR A DISTANCE AS POSSIBLE. DO NOT USE WATER DIRECTLY ON MATERIAL. IF LARGE AMOUNTS OF COMBUSTIBLE MATERIALS ARE INVOLVED, USE WATER SPRAY AND FOG IN FLOODING AMOUNTS. USE WATER SPRAY TO ABSORB FLAMMABLE, CORROSIVE VAPORS. AVOID BREATHING CORROSIVE VAPORS; KEEP UPWIND.

WATER MAY BE INEFFECTIVE (NFPA 325M, FIRE HAZARD PROPERTIES OF FLAMMABLE LIQUIDS, GASES, AND VOLATILE SOLIDS, 1991)

TOXICITY

PIPERIDINE:
IRRITATION DATA: 100 UG/24 HOURS OPEN SKIN-RABBIT; 5 MG/24 HOURS SKIN-RABBIT SEVERE; 250 UG/24 HOURS EYE-RABBIT SEVERE.
TOXICITY DATA: 4000 PPM/4 HOURS INHALATION-RAT LCLO; 6000 MG/M3/2 HOURS INHALATION-MOUSE LC50; 6500 MG/M3 INHALATION-MAMMAL LD50; 320 MG/KG

SKIN-RABBIT LD50; 400 MG/KG ORAL-RAT LD50; 145 MG/KG ORAL-RABBIT LD50; 30 MG/KG ORAL-MOUSE LD50; 22400 UG/KG ORAL-MAMMAL LD50; 300 MG/KG SUBCUTANEOUS-RABBIT LDLO; 460 MG/KG SUBCUTANEOUS-MOUSE LDLO; 160 MG/KG INTRAVENOUS-RABBIT LDLO; 50 MG/KG INTRAPERITONEAL-MOUSE LDLO; MUTAGENIC DATA (RTECS); REPRODUCTIVE EFFECTS DATA (RTECS).

CARCINOGEN STATUS: NONE.

LOCAL EFFECTS: CORROSIVE- INHALATION, SKIN, EYE, INGESTION.

ACUTE TOXICTY LEVEL: TOXIC BY INHALATION, DERMAL ABSORPTION, INGESTION.

TARGET EFFECTS: POISONING MAY AFFECT THE NERVOUS SYSTEM.

ADDITIONAL DATA: INTRAVENOUS ADMINISTRATION HAD AN INITIAL STIMULANT AND SECONDARY DEPRESSANT EFFECT ON SYMPATHETIC AND PARASYMPATHETIC GANGLIA.

HEALTH EFFECTS AND FIRST AID

INHALATION:
PIPERIDINE:
CORROSIVE/TOXIC.

ACUTE EXPOSURE- MAY CAUSE IRRITATION AND BURNS OF THE NOSE, THROAT AND MUCOUS MEMBRANES OF THE RESPIRATORY TRACT. ABSORPTION MAY CAUSE EFFECTS ON THE NERVOUS SYSTEM. ALSO, SORE THROAT, COUGH, LABORED BREATHING, DULLNESS AND PULMONARY EDEMA WHICH MAY BE DELAYED UP TO 2 DAYS MAY OCCUR. THE REPORTED LETHAL DOSE IN MICE IS 6000 MG/M3/2 HOURS.

CHRONIC EXPOSURE- REPEATED OR PROLONGED EXPOSURE MAY CAUSE PULMONARY EDEMA. REPRODUCTIVE EFFECTS HAVE BEEN REPORTED IN ANIMALS.

FIRST AID- REMOVE FROM EXPOSURE AREA TO FRESH AIR IMMEDIATELY. IF BREATHING HAS STOPPED, GIVE ARTIFICIAL RESPIRATION. MAINTAIN AIRWAY AND BLOOD PRESSURE AND ADMINISTER OXYGEN IF AVAILABLE. KEEP AFFECTED PERSON WARM AND AT REST. TREAT SYMPTOMATICALLY AND SUPPORTIVELY. ADMINISTRATION OF OXYGEN SHOULD BE PERFORMED BY QUALIFIED PERSONNEL. GET MEDICAL ATTENTION IMMEDIATELY.

SKIN CONTACT:
PIPERIDINE:
CORROSIVE/TOXIC.

ACUTE EXPOSURE- MAY CAUSE SEVERE IRRITATION AND BURNS. ABSORPTION MAY PRODUCE EFFECTS ON THE NERVOUS SYSTEM. THE MEAN LETHAL DOSE APPLIED TO RABBIT SKIN WAS 320 MG/KG. NO EFFECTS FROM SKIN ABSORPTION WERE REPORTED.

CHRONIC EXPOSURE- REPEATED OR PROLONGED CONTACT MAY CAUSE DERMATITIS.

FIRST AID- REMOVE CONTAMINATED CLOTHING AND SHOES IMMEDIATELY. WASH AFFECTED AREA WITH SOAP OR MILD DETERGENT AND LARGE AMOUNTS OF WATER UNTIL NO EVIDENCE OF CHEMICAL REMAINS (AT LEAST 15-20 MINUTES). IN CASE OF CHEMICAL BURNS, COVER AREA WITH STERILE, DRY DRESSING. BANDAGE SECURELY, BUT NOT TOO TIGHTLY. GET MEDICAL ATTENTION IMMEDIATELY.

EYE CONTACT:
PIPERIDINE:
CORROSIVE.

ACUTE EXPOSURE- MAY CAUSE IRRITATION AND BURNS WITH POSSIBLE PERMANENT DAMAGE. SEVERE INJURY OF THE CORNEA OF RABBITS RATING A 9 ON A SCALE OF 1 TO 10 HAS BEEN REPORTED.

CHRONIC EXPOSURE- PROLONGED CONTACT MAY CAUSE CONJUNCTIVITIS.

FIRST AID- WASH EYES IMMEDIATELY WITH LARGE AMOUNTS OF WATER, OCCASIONALLY LIFTING UPPER AND LOWER LIDS, UNTIL NO EVIDENCE OF CHEMICAL REMAINS (AT LEAST 15-20 MINUTES). CONTINUE IRRIGATING WITH NORMAL SALINE UNTIL THE PH HAS RETURNED TO NORMAL (30-60 MINUTES). COVER WITH STERILE BANDAGES. GET

MEDICAL ATTENTION IMMEDIATELY.

INGESTION:
PIPERIDINE:
CORROSIVE/TOXIC.
ACUTE EXPOSURE- MAY CAUSE SORE THROAT, IRRITATION AND BURNS. ABSORPTION MAY PRODUCE EFFECTS ON THE NERVOUS SYSTEM. EFFECTS REPORTED IN ANIMALS INCLUDE WEAKNESS, RESPIRATORY DISTRESS AND CONVULSIONS. THE MEAN LETHAL DOSE REPORTED IN RATS WAS 400 MG/KG.
CHRONIC EXPOSURE- NO DATA AVAILABLE.

FIRST AID- TREAT SYMPTOMATICALLY AND SUPPORTIVELY. IF PERSON IS CONSCIOUS AND ABLE TO SWALLOW, GIVE LARGE AMOUNTS OF WATER OR MILK TO DILUTE SUBSTANCE. GET MEDICAL ATTENTION IMMEDIATELY. GASTRIC LAVAGE PERFORMED BY QUALIFIED MEDICAL PERSONNEL MIGHT BE ADVISABLE IF THERE ARE NO SIGNS OF PERFORATION FROM THE INGESTION OF A CORROSIVE SUBSTANCE. IF VOMITING OCCURS, KEEP HEAD BELOW HIPS TO HELP PREVENT ASPIRATION.

ANTIDOTE:
NO SPECIFIC ANTIDOTE. TREAT SYMPTOMATICALLY AND SUPPORTIVELY.

REACTIVITY

REACTIVITY:
WHEN DISSOLVED IN WATER, THE SUBSTANCE IS A STRONG BASE, IT REACTS VIOLENTLY WITH ACIDS AND IS CORROSIVE TOWARDS ALUMINUM, ZINC AND OTHER METALS. REACTS VIOLENTLY WITH OXIDANTS.

INCOMPATIBILITIES:
PIPERIDINE:
ACIDS: INCOMPATABLE.
ACID ANHYDRIDES: INCOMPATABLE.
ACID CHLORIDES: INCOMPATABLE.
ALUMINUM: CORROSIVE.
CARBON DIOXIDE: INCOMPATABLE.
DICYANOFURAZAN: INSTANTANEOUSLY EXPLOSIVE.
N-NITROSOACETANILIDE: THE DRY ANILIDE MAY EXPLODE ON CONTACT WITH A DROP OF PIPERIDINE.
1-PERCHLORYLPIPERIDINE: EXPLOSION ON CONTACT.
OXIDIZERS: VIGOROUS REACTION.
ZINC: CORROSIVE.

DECOMPOSITION:
THERMAL DECOMPOSITION PRODUCTS MAY INCLUDE TOXIC OXIDES OF CARBON AND NITROGEN.

POLYMERIZATION:
HAZARDOUS POLYMERIZATION HAS NOT BEEN REPORTED TO OCCUR UNDER NORMAL TEMPERATURES AND PRESSURES.

STORAGE AND DISPOSAL

OBSERVE ALL FEDERAL, STATE AND LOCAL REGULATIONS WHEN STORING OR DISPOSING OF THIS SUBSTANCE. FOR ASSISTANCE, CONTACT THE DISTRICT DIRECTOR OF THE ENVIRONMENTAL PROTECTION AGENCY.

STORAGE

BONDING AND GROUNDING: SUBSTANCES WITH LOW ELECTROCONDUCTIVITY, WHICH MAY BE IGNITED BY ELECTROSTATIC SPARKS, SHOULD BE STORED IN CONTAINERS WHICH MEET THE BONDING AND GROUNDING GUIDELINES SPECIFIED IN NFPA 77-1983, RECOMMENDED PRACTICE ON STATIC ELECTRICITY.

KEEP IN A TIGHTLY CLOSED CONTAINER. STORE IN A COOL, DRY, VENTILATED AREA.

STORE AWAY FROM INCOMPATIBLE SUBSTANCES.

STORE IN ACCORDANCE WITH 29 CFR 1910.106.

THRESHOLD PLANNING QUANTITY (TPQ):
THE SUPERFUND AMENDMENTS AND REAUTHORIZATION ACT (SARA) SECTION 302 REQUIRES THAT EACH FACILITY WHERE ANY EXTREMELY HAZARDOUS SUBSTANCE IS PRESENT IN A QUANTITY EQUAL TO OR GREATER THAN THE TPQ ESTABLISHED FOR THAT SUBSTANCE NOTIFY THE STATE EMERGENCY RESPONSE COMMISSION FOR THE STATE IN WHICH IT IS LOCATED. SECTION 303 OF SARA REQUIRES THESE FACILITIES TO PARTICIPATE IN LOCAL EMERGENCY RESPONSE PLANNING (40 CFR 355.30).

DISPOSAL

DISPOSAL MUST BE IN ACCORDANCE WITH STANDARDS APPLICABLE TO GENERATORS OF HAZARDOUS WASTE, 40 CFR 262. EPA HAZARDOUS WASTE NUMBERS, D001 AND D003.
100 POUND CERCLA SECTION 103 REPORTABLE QUANTITY.

CONDITIONS TO AVOID

AVOID CONTACT WITH HEAT, SPARKS, FLAMES OR OTHER IGNITION SOURCES. VAPORS MAY BE EXPLOSIVE. MATERIAL IS CORROSIVE; AVOID CONTACT WITH SKIN OR EYES. DO NOT ALLOW CONTAMINATION OF WATER SOURCES.

SPILL AND LEAK PROCEDURES

OCCUPATIONAL SPILL:
SHUT OFF IGNITION SOURCES. DO NOT TOUCH SPILLED MATERIAL. STOP LEAK IF YOU CAN DO IT WITHOUT RISK. USE WATER SPRAY TO REDUCE VAPORS. DO NOT GET WATER INSIDE CONTAINER. FOR SMALL SPILLS, TAKE UP WITH SAND OR OTHER ABSORBENT MATERIAL AND PLACE INTO CONTAINERS FOR LATER DISPOSAL. FOR LARGER SPILLS, DIKE FAR AHEAD OF SPILL FOR LATER DISPOSAL. NO SMOKING, FLAMES OR FLARES IN HAZARD AREA. KEEP UNNECESSARY PEOPLE AWAY; ISOLATE HAZARD AREA AND DENY ENTRY.

REPORTABLE QUANTITY (RQ): 1 POUND
THE SUPERFUND AMENDMENTS AND REAUTHORIZATION ACT (SARA) SECTION 304 REQUIRES THAT A RELEASE EQUAL TO OR GREATER THAN THE REPORTABLE QUANTITY FOR THIS SUBSTANCE BE IMMEDIATELY REPORTED TO THE LOCAL EMERGENCY PLANNING COMMITTEE AND THE STATE EMERGENCY RESPONSE COMMISSION (40 CFR 355.40). IF THE RELEASE OF THIS SUBSTANCE IS REPORTABLE UNDER CERCLA SECTION 103, THE NATIONAL RESPONSE CENTER MUST BE NOTIFIED IMMEDIATELY AT (800) 424-8802 OR (202) 426-2675 IN THE METROPOLITAN WASHINGTON, D.C. AREA (40 CFR 302.6).

PROTECTIVE EQUIPMENT

VENTILATION:
PROVIDE LOCAL EXHAUST OR PROCESS ENCLOSURE VENTILATION. VENTILATION EQUIPMENT MUST BE EXPLOSION-PROOF.

RESPIRATOR:
THE FOLLOWING RESPIRATORS ARE RECOMMENDED BASED ON INFORMATION FOUND IN THE PHYSICAL DATA, TOXICITY AND HEALTH EFFECTS SECTIONS. THEY ARE RANKED IN ORDER FROM MINIMUM TO MAXIMUM RESPIRATORY PROTECTION.
THE SPECIFIC RESPIRATOR SELECTED MUST BE BASED ON CONTAMINATION LEVELS FOUND IN THE WORK PLACE, MUST BE BASED ON THE SPECIFIC OPERATION, MUST NOT EXCEED THE WORKING LIMITS OF THE RESPIRATOR AND MUST BE JOINTLY APPROVED BY THE NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH AND THE MINE SAFETY AND HEALTH ADMINISTRATION (NIOSH-MSHA).

ANY TYPE 'C' SUPPLIED-AIR RESPIRATOR WITH A FULL FACEPIECE OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE OR WITH A FULL FACEPIECE, HELMET OR HOOD OPERATED IN CONTINUOUS-FLOW MODE.

ANY SELF-CONTAINED BREATHING APPARATUS WITH A FULL FACEPIECE OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE.

FOR FIREFIGHTING AND OTHER IMMEDIATELY DANGEROUS TO LIFE OR HEALTH CONDITIONS:

ANY SELF-CONTAINED BREATHING APPARATUS THAT HAS A FULL FACEPIECE AND IS OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE.

ANY SUPPLIED-AIR RESPIRATOR THAT HAS A FULL FACEPIECE AND IS OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE IN COMBINATION WITH AN AUXILIARY SELF-CONTAINED BREATHING APPARATUS OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE.

CLOTHING:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE (IMPERVIOUS) CLOTHING AND EQUIPMENT TO PREVENT ANY POSSIBILITY OF SKIN CONTACT WITH THIS SUBSTANCE.

GLOVES:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE GLOVES TO PREVENT CONTACT WITH THIS SUBSTANCE.

EYE PROTECTION:
EMPLOYEE MUST WEAR SPLASH-PROOF OR DUST-RESISTANT SAFETY GOGGLES AND A FACESHIELD TO PREVENT CONTACT WITH THIS SUBSTANCE.

EMERGENCY WASH FACILITIES:
WHERE THERE IS ANY POSSIBILITY THAT AN EMPLOYEE'S EYES AND/OR SKIN MAY BE EXPOSED TO THIS SUBSTANCE, THE EMPLOYER SHOULD PROVIDE AN EYE WASH FOUNTAIN AND QUICK DRENCH SHOWER WITHIN THE IMMEDIATE WORK AREA FOR EMERGENCY USE.

CREATION DATE: 07/31/91 REVISION DATE: 05/21/92

MATERIAL SAFETY DATA SHEET

APPLIED BIOSYSTEMS
850 LINCOLN CENTRE DRIVE
FOSTER CITY, CA 94404
(415) 570-6667 (USA)
0925-825650 (UK)

24 HOUR EMERGENCY RESPONSE
NUMBER: 615-366-2000

SUBSTANCE IDENTIFICATION

CAS NUMBER: 109-99-9
RTECS NUMBER: LU5950000

SUBSTANCE: TETRAHYDROFURAN, STABILIZED

TRADE NAMES/SYNONYMS:
ABI MSDS PART # 902034; P/N 401255; CYCLOTETRAMETHYLENE OXIDE; FURANIDINE; OXACYCLOPENTANE; OXOLANE; TETRAMETHYLENE OXIDE; THF; RCRA U213; TETRAHYDROFURAN; FURAN, TETRAHYDRO-; TETRAHYDROFURAN, BHT STABILIZED; BUTANE, ALPHA, DELTA-OXIDE; STCC 4908290; UN 2056; C4H8O;

CHEMICAL FAMILY:
FURAN DERIVATIVE

MOLECULAR FORMULA: C4-H8-O

MOLECULAR WEIGHT: 72.10

CERCLA RATINGS (SCALE 0-3): HEALTH=3 FIRE=3 REACTIVITY=1 PERSISTENCE=1
NFPA RATINGS (SCALE 0-4): HEALTH=2 FIRE=3 REACTIVITY=1

CERCLA RATINGS (SCALE 0-3):HEALTH=3FIRE=3REACTIVITY=1PERSISTENCE=1
NFPA RATINGS (SCALE 0-4):HEALTH=2FIRE=3REACTIVITY=1

COMPONENTS AND CONTAMINANTS

COMPONENT: TETRAHYDROFURAN PERCENT: >99
CAS# 109-99-9

OTHER CONTAMINANTS: MAY CONTAIN TRACES OF A PEROXIDATION INHIBITOR.

EXPOSURE LIMITS:
TETRAHYDROFURAN:
200 PPM (590 MG/M3) OSHA TWA; 250 PPM (737 MG/M3) OSHA STEL
200 PPM (590 MG/M3) ACGIH TWA; 250 PPM (737 MG/M3) ACGIH STEL
200 PPM (590 MG/M3) NIOSH RECOMMENDED TWA;
250 PPM (737 MG/M3) NIOSH RECOMMENDED STEL
200 PPM (590 MG/M3) DFG MAK TWA;
1000 PPM (2950 MG/M3) DFG MAK 30 MINUTE PEAK, AVERAGE VALUE, 2 TIMES/SHIFT

MEASUREMENT METHOD: CHARCOAL TUBE; CARBON DISULFIDE; GAS CHROMATOGRAPHY WITH FLAME IONIZATION DETECTION; (NIOSH VOL. III # 1609).

1000 POUND CERCLA SECTION 103 REPORTABLE QUANTITY

PHYSICAL DATA

DESCRIPTION: COLORLESS, VOLATILE, MOBILE LIQUID WITH AN ETHER-LIKE ODOR.

BOILING POINT: 153 F (67 C) MELTING POINT: -162 F (-108 C)

SPECIFIC GRAVITY: 0.8892 VAPOR PRESSURE: 145 MMHG @ 20 C

EVAPORATION RATE: (N-BUTYL ACETATE=1) 14.5 SOLUBILITY IN WATER: COMPLETE

ODOR THRESHOLD: 20-50 PPM VAPOR DENSITY: 2.5

SOLVENT SOLUBILITY: SOLUBLE IN BENZENE, ALCOHOLS, ETHERS, KETONES, ESTERS, HYDROCARBONS.

VISCOSITY: 0.5 CPS @ 20 C

FIRE AND EXPLOSION DATA

FIRE AND EXPLOSION HAZARD:
DANGEROUS FIRE HAZARD WHEN EXPOSED TO HEAT OR FLAME.

VAPOR-AIR MIXTURES ARE EXPLOSIVE.

VAPORS ARE HEAVIER THAN AIR AND MAY TRAVEL A CONSIDERABLE DISTANCE TO A SOURCE OF IGNITION AND FLASH BACK.

FLASH POINT: 6 F (-14 C) (CC) UPPER EXPLOSIVE LIMIT: 11.8%

LOWER EXPLOSIVE LIMIT: 2% AUTOIGNITION TEMP.: 610 F (321 C)

FLAMMABILITY CLASS(OSHA): IB

FIREFIGHTING MEDIA:
DRY CHEMICAL, CARBON DIOXIDE, WATER SPRAY OR ALCOHOL-RESISTANT FOAM (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

FOR LARGER FIRES, USE WATER SPRAY, FOG OR ALCOHOL-RESISTANT FOAM (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

ALCOHOL FOAM
(NFPA 325M, FIRE HAZARD PROPERTIES OF FLAMMABLE LIQUIDS, GASES, AND VOLATILE SOLIDS, 1991).

FIREFIGHTING:
MOVE CONTAINER FROM FIRE AREA IF YOU CAN DO IT WITHOUT RISK. APPLY COOLING WATER TO SIDES OF CONTAINERS THAT ARE EXPOSED TO FLAMES UNTIL WELL AFTER FIRE IS OUT. STAY AWAY FROM ENDS OF TANKS. FOR MASSIVE FIRE IN CARGO AREA, USE UNMANNED HOSE HOLDER OR MONITOR NOZZLES; IF THIS IS IMPOSSIBLE, WITHDRAW FROM AREA AND LET FIRE BURN. WITHDRAW IMMEDIATELY IN CASE OF RISING SOUND FROM VENTING SAFETY DEVICE OR ANY DISCOLORATION OF TANK DUE TO FIRE. ISOLATE FOR 1/2 MILE IN ALL DIRECTIONS IF TANK, RAIL CAR OR TANK TRUCK IS INVOLVED IN FIRE (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5, GUIDE PAGE 26).

EXTINGUISH ONLY IF FLOW CAN BE STOPPED; USE WATER IN FLOODING AMOUNTS AS FOG, SOLID STREAMS MAY NOT BE EFFECTIVE. COOL CONTAINERS WITH FLOODING QUANTITIES OF WATER, APPLY FROM AS FAR A DISTANCE AS POSSIBLE. AVOID BREATHING TOXIC VAPORS, KEEP UPWIND. EVACUATE TO A RADIUS OF 1500 FEET FOR UNCONTROLLABLE FIRES. CONSIDER EVACUATION OF DOWNWIND AREA IF MATERIAL IS LEAKING.

WATER MAY BE INEFFECTIVE (NFPA 325M, FIRE HAZARD PROPERTIES OF FLAMMABLE LIQUIDS, GASES, AND VOLATILE SOLIDS, 1991)

TOXICITY

TETRAHYDROFURAN:
TOXICITY DATA: 25000 PPM INHALATION-HUMAN TCLO; 21000 PPM/3 HOURS INHALATION-RAT LC50; 24000 MG/M3/2 HOURS INHALATION-MOUSE LCLO; 1650 MG/KG ORAL-RAT LD50; 2300 MG/KG ORAL-MOUSE LD50; 2300 MG/KG ORAL-GUINEA PIG LD50; 2900 MG/KG INTRAPERITONEAL-RAT LD50; 1900 MG/KG INTRAPERITONEAL-MOUSE LD50; 500 MG/KG INTRAPERITONEAL-GUINEA PIG LDLO; MUTAGENIC DATA (RTECS).
CARCINOGEN STATUS: NONE.
LOCAL EFFECTS: IRRITANT- INHALATION, EYE.
ACUTE TOXICITY LEVEL: MODERATELY TOXIC BY INGESTION, SLIGHTLY TOXIC BY INHALATION.
TARGET EFFECTS: CENTRAL NERVOUS SYSTEM DEPRESSANT; POISONING MAY AFFECT THE LIVER AND KIDNEYS.
AT INCREASED RISK FROM EXPOSURE: PERSONS WITH IMPAIRED LIVER, KIDNEY, OR PULMONARY FUNCTION; EYE OR SKIN DISORDERS.
ADDITIONAL DATA: ALCOHOL MAY ENHANCE THE TOXIC EFFECTS.

HEALTH EFFECTS AND FIRST AID

INHALATION:
TETRAHYDROFURAN:
IRRITANT/NARCOTIC. 20,000 PPM IMMEDIATELY DANGEROUS TO LIFE OR HEALTH.
ACUTE EXPOSURE- TECHNICIANS WORKING WITH TETRAHYDROFURAN EXPERIENCED SEVERE OCCIPITAL HEADACHES AND DULLNESS. OTHER EFFECTS REPORTED FROM HIGH VAPOR CONCENTRATIONS INCLUDE MUCOUS MEMBRANE IRRITATION WITH SORE THROAT AND COUGHING, NAUSEA, CENTRAL NERVOUS SYSTEM DEPRESSION WITH WEAKNESS, FATIGUE, UNCONSCIOUSNESS AND POSSIBLY ASPHYXIATION. CONCENTRATIONS ABOVE 25,000 PPM PRODUCED ANESTHESIA WITH PROLONGED INDUCTION, PROFUSE SALIVATION, VOMITING, MARKED FALL IN BLOOD PRESSURE, POOR MUSCULAR RELAXATION, AND STRONG RESPIRATORY STIMULATION IN EXPERIMENTAL ANIMALS.
CHRONIC EXPOSURE- ANIMALS EXPOSED TO OVER 3000 PPM FOR 20 DAYS EXHIBITED IRRITATION OF THE UPPER RESPIRATORY TRACT. LIVER AND KIDNEY DAMAGE WAS OBSERVED, BUT MAY HAVE BEEN DUE TO IMPURITIES. OTHER REPORTED EFFECTS FROM REPEATED EXPOSURE INCLUDE DISCHARGE OR BLEEDING IN THE NASAL MUCOSA, A CATALEPTOID POSTURE, COMA AND CLONIC MUSCLE SPASMS. LEVELS OF 5000 PPM FOR 91 DAYS PRODUCED ACANTHOSIS AND SUPPURATIVE INFLAMMATION OF THE FORESTOMACH, MINIMAL CENTRILOBULAR HYPERTROPHY OF THE LIVER AND ATROPHY OF THE UTERUS AND DEGENERATION OF THE ADRENAL CORTEX.

FIRST AID- REMOVE FROM EXPOSURE AREA TO FRESH AIR IMMEDIATELY. IF BREATHING HAS STOPPED, PERFORM ARTIFICIAL RESPIRATION. KEEP PERSON WARM AND AT REST. TREAT SYMPTOMATICALLY AND SUPPORTIVELY. GET MEDICAL ATTENTION IMMEDIATELY.

SKIN CONTACT:
TETRAHYDROFURAN:
ACUTE EXPOSURE- LIQUID APPLIED TO THE SKIN OF 196 PERSONS WAS ESSENTIALLY NONIRRITATING. HOWEVER, AQUEOUS SOLUTIONS IN CONCENTRATIONS ABOVE 20% HAVE BEEN REPORTED TO CAUSE IRRITATION WHEN APPLIED TO RABBIT SKIN. SKIN ABSORPTION MAY OCCUR.
CHRONIC EXPOSURE- REPEATED OR PROLONGED EXPOSURE MAY CAUSE IRRITATION, DEHYDRATION AND DEFATTING, AND SUBSEQUENT DERMATITIS.

FIRST AID- REMOVE CONTAMINATED CLOTHING AND SHOES IMMEDIATELY. WASH AFFECTED AREA WITH SOAP OR MILD DETERGENT AND LARGE AMOUNTS OF WATER UNTIL NO EVIDENCE OF CHEMICAL REMAINS (APPROXIMATELY 15-20 MINUTES). GET MEDICAL ATTENTION IMMEDIATELY.

EYE CONTACT:
TETRAHYDROFURAN:
IRRITANT.
ACUTE EXPOSURE- VAPORS MAY CAUSE IRRITATION, REDNESS, TEARING, AND BLURRED VISION. THE LIQUID MAY CAUSE SEVERE IRRITATION OR POSSIBLY BURNS.
CHRONIC EXPOSURE- RATS EXPOSED TO 5000 PPM SHOWED MARKED EDEMA OR OPACITY OF THE CORNEA.

FIRST AID- WASH EYES IMMEDIATELY WITH LARGE AMOUNTS OF WATER OR NORMAL SALINE, OCCASIONALLY LIFTING UPPER AND LOWER LIDS, UNTIL NO EVIDENCE OF CHEMICAL REMAINS (APPROXIMATELY 15-20 MINUTES). GET MEDICAL ATTENTION IMMEDIATELY.

INGESTION:
TETRAHYDROFURAN:
ACUTE EXPOSURE- MAY CAUSE GASTROINTESTINAL IRRITATION, SORE THROAT, ABDOMINAL PAIN, NAUSEA, VOMITING, DIARRHEA, AND DULLNESS. ASPIRATION MAY OCCUR AND CAUSE SEVERE LUNG IRRITATION AND POSSIBLY DELAYED CHEMICAL PNEUMONITIS.
CHRONIC EXPOSURE- REPEATED AND PROLONGED EXPOSURE HAVE BEEN REPORTED TO CAUSE LIVER AND KIDNEY DAMAGE.

FIRST AID- IF THE PERSON IS CONSCIOUS AND NOT CONVULSING, INDUCE EMESIS BY GIVING SYRUP OF IPECAC FOLLOWED BY WATER. (IF VOMITING OCCURS KEEP THE HEAD BELOW THE HIPS TO PREVENT ASPIRATION). REPEAT IN 20 MINUTES IF NOT EFFECTIVE INITIALLY. GIVE ACTIVATED CHARCOAL. IN PATIENTS WITH DEPRESSED RESPIRATION OR IF EMESIS IS NOT PRODUCED, PERFORM GASTRIC LAVAGE CAUTIOUSLY (DREISBACH, HANDBOOK OF POISONING, 12TH ED.). TREAT SYMPTOMATICALLY AND SUPPORTIVELY. GASTRIC LAVAGE SHOULD BE PERFORMED BY QUALIFIED MEDICAL PERSONNEL. GET MEDICAL ATTENTION IMMEDIATELY.

ANTIDOTE:
NO SPECIFIC ANTIDOTE. TREAT SYMPTOMATICALLY AND SUPPORTIVELY.

REACTIVITY

REACTIVITY:
STABLE UNDER NORMAL TEMPERATURES AND PRESSURES IF PEROXIDATION IS INHIBITED. IF UNINHIBITED, MAY FORM EXPLOSIVE ORGANIC PEROXIDES WHEN EXPOSED TO AIR. IF DISTILLED OR EVAPORATED TO DRYNESS, AN EXPLOSION MAY OCCUR.

INCOMPATIBILITIES:
TETRAHYDROFURAN:
ACIDS: REACTION AND POSSIBLE POLYMERIZATION.
BASES: INCOMPATIBLE.
BORANE-TETRAHYDROFURAN COMPLEX: POSSIBLE EXPLOSION ON STORAGE.
BROMINE: VIGOROUS REACTION.
HAFNIUM TETRACHLORIDE: VIOLENT EXOTHERMIC REACTION.
LITHIUM ALUMINUM ALLOYS: EXPLOSION.
LITHIUM ALUMINUM HYDRIDE (LITHIUM TETRAHYDROALUMINATE): POSSIBLE IGNITION.
METALLIC HYDRIDES: POSSIBLE EXPLOSION WHEN HEATED.
OXIDIZERS (STRONG): FIRE AND EXPLOSION HAZARD.
PLASTICS, RUBBER, COATINGS: SOME FORMS ATTACKED.
POTASSIUM DIOXIDE AND 2-AMINOPHENOL: POSSIBLE EXPLOSION.
POTASSIUM HYDROXIDE: POSSIBLE EXPLOSION IN PRESENCE OF PEROXIDE CONTAMINANTS.

SODIUM HYDROXIDE: POSSIBLE EXPLOSION IN PRESENCE OF PEROXIDE CONTAMINANTS.
SODIUM TETRAHYDROALUMINATE: POSSIBLE VIOLENT EXPLOSION.
TITANIUM TETRACHLORIDE: VIOLENT EXOTHERMIC REACTION.
ZIRCONIUM TETRACHLORIDE: VIOLENT EXOTHERMIC REACTION.

DECOMPOSITION:
THERMAL DECOMPOSITION PRODUCTS MAY INCLUDE TOXIC OXIDES OF CARBON.

POLYMERIZATION:
MAY POLYMERIZE EXOTHERMICALLY IN CONTACT WITH SOME ACIDS.

STORAGE AND DISPOSAL

OBSERVE ALL FEDERAL, STATE AND LOCAL REGULATIONS WHEN STORING OR DISPOSING OF THIS SUBSTANCE. FOR ASSISTANCE, CONTACT THE DISTRICT DIRECTOR OF THE ENVIRONMENTAL PROTECTION AGENCY.

STORAGE

STORE IN ACCORDANCE WITH 29 CFR 1910.106.

BONDING AND GROUNDING: SUBSTANCES WITH LOW ELECTROCONDUCTIVITY, WHICH MAY BE IGNITED BY ELECTROSTATIC SPARKS, SHOULD BE STORED IN CONTAINERS WHICH MEET THE BONDING AND GROUNDING GUIDELINES SPECIFIED IN NFPA 77-1983, RECOMMENDED PRACTICE ON STATIC ELECTRICITY.

STORE IN A COOL, DRY, WELL-VENTILATED LOCATION. STORE AWAY FROM HEAT, OXIDIZING MATERIALS, AND SUNLIGHT. OUTSIDE OR DETACHED STORAGE IS PREFERRED. INSIDE STORAGE SHOULD BE IN A STANDARD FLAMMABLE LIQUIDS STORAGE WAREHOUSE, ROOM, OR CABINET. (NFPA 49, HAZARDOUS CHEMICALS DATA, 1991).

STORAGE TEMPERATURE: ROOM TEMPERATURE IN A WELL-VENTILATED AREA.
SHELF LIFE: INDEFINITE.

STORE AWAY FROM INCOMPATIBLE SUBSTANCES.

DISPOSAL

DISPOSAL MUST BE IN ACCORDANCE WITH STANDARDS APPLICABLE TO GENERATORS OF HAZARDOUS WASTE, 40CFR 262. EPA HAZARDOUS WASTE NUMBER U213.

CONDITIONS TO AVOID

AVOID CONTACT WITH HEAT, SPARKS, FLAMES, OR OTHER SOURCES OF IGNITION. VAPORS MAY BE EXPLOSIVE AND POISONOUS; DO NOT ALLOW UNNECESSARY PERSONNEL IN AREA. DO NOT OVERHEAT CONTAINERS; CONTAINERS MAY VIOLENTLY RUPTURE AND TRAVEL A CONSIDERABLE DISTANCE IN HEAT OF FIRE.

SPILL AND LEAK PROCEDURES

OCCUPATIONAL SPILL:
SHUT OFF IGNITION SOURCES. STOP LEAK IF YOU CAN DO IT WITHOUT RISK. USE WATER SPRAY TO REDUCE VAPORS. FOR SMALL SPILLS, TAKE UP WITH SAND OR OTHER ABSORBENT MATERIAL AND PLACE INTO CONTAINERS FOR LATER DISPOSAL. FOR LARGER SPILLS, DIKE FAR AHEAD OF SPILL FOR LATER DISPOSAL. NO SMOKING, FLAMES OR FLARES IN HAZARD AREA. KEEP UNNECESSARY PEOPLE AWAY; ISOLATE HAZARD AREA AND DENY ENTRY.

REPORTABLE QUANTITY (RQ): 1000 POUNDS
THE SUPERFUND AMENDMENTS AND REAUTHORIZATION ACT (SARA) SECTION 304 REQUIRES THAT A RELEASE EQUAL TO OR GREATER THAN THE REPORTABLE QUANTITY FOR THIS SUBSTANCE BE IMMEDIATELY REPORTED TO THE LOCAL EMERGENCY PLANNING COMMITTEE AND THE STATE EMERGENCY RESPONSE COMMISSION (40 CFR 355.40). IF THE RELEASE OF THIS SUBSTANCE IS REPORTABLE UNDER CERCLA SECTION 103, THE NATIONAL RESPONSE CENTER MUST BE NOTIFIED IMMEDIATELY AT (800) 424-8802 OR (202) 426-2675 IN THE METROPOLITAN WASHINGTON, D.C. AREA (40 CFR 302.6).

PROTECTIVE EQUIPMENT

VENTILATION:
PROVIDE GENERAL DILUTION VENTILATION TO MEET PUBLISHED EXPOSURE LIMITS. VENTILATION EQUIPMENT MUST BE EXPLOSION-PROOF.

RESPIRATOR:
THE FOLLOWING RESPIRATORS AND MAXIMUM USE CONCENTRATIONS ARE RECOMMENDATIONS BY THE U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, NIOSH POCKET GUIDE TO CHEMICAL HAZARDS; NIOSH CRITERIA DOCUMENTS OR BY THE U.S. DEPARTMENT OF LABOR, 29 CFR 1910 SUBPART Z.
THE SPECIFIC RESPIRATOR SELECTED MUST BE BASED ON CONTAMINATION LEVELS FOUND IN THE WORK PLACE, MUST NOT EXCEED THE WORKING LIMITS OF THE RESPIRATOR AND BE JOINTLY APPROVED BY THE NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH AND THE MINE SAFETY AND HEALTH ADMINISTRATION (NIOSH-MSHA).

TETRAHYDROFURAN:

1000 PPM-	ANY POWERED AIR-PURIFYING RESPIRATOR WITH ORGANIC VAPOR CARTRIDGE(S). ANY CHEMICAL CARTRIDGE RESPIRATOR WITH A FULL FACEPIECE AND ORGANIC VAPOR CARTRIDGE(S).
5000 PPM-	ANY SUPPLIED-AIR RESPIRATOR OPERATED IN A CONTINUOUS FLOW MODE.
10,000 PPM-	ANY AIR-PURIFYING FULL FACEPIECE RESPIRATOR (GAS MASK) WITH A CHIN-STYLE OR FRONT- OR BACK-MOUNTED ORGANIC VAPOR CANISTER. ANY SELF-CONTAINED BREATHING APPARATUS WITH A FULL FACEPIECE. ANY SUPPLIED-AIR RESPIRATOR WITH A FULL FACEPIECE.
20,000 PPM-	ANY SUPPLIED-AIR RESPIRATOR WITH A FULL FACEPIECE AND OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE.
ESCAPE-	ANY AIR-PURIFYING FULL FACEPIECE RESPIRATOR (GAS MASK) WITH A CHIN-STYLE OR FRONT- OR BACK-MOUNTED ORGANIC VAPOR CANISTER. ANY APPROPRIATE ESCAPE-TYPE SELF-CONTAINED BREATHING APPARATUS.

FOR FIREFIGHTING AND OTHER IMMEDIATELY DANGEROUS TO LIFE OR HEALTH CONDITIONS:

ANY SELF-CONTAINED BREATHING APPARATUS THAT HAS A FULL FACEPIECE AND IS OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE.

ANY SUPPLIED-AIR RESPIRATOR THAT HAS A FULL FACEPIECE AND IS OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE IN COMBINATION WITH AN AUXILIARY SELF-CONTAINED BREATHING APPARATUS OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE.

CLOTHING:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE (IMPERVIOUS) CLOTHING AND EQUIPMENT TO PREVENT REPEATED OR PROLONGED SKIN CONTACT WITH THIS SUBSTANCE.

GLOVES:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE GLOVES TO PREVENT CONTACT WITH THIS SUBSTANCE.

EYE PROTECTION:
EMPLOYEE MUST WEAR SPLASH-PROOF OR DUST-RESISTANT SAFETY GOGGLES TO PREVENT EYE CONTACT WITH THIS SUBSTANCE.

EMERGENCY EYE WASH: WHERE THERE IS ANY POSSIBILITY THAT AN EMPLOYEE'S EYES MAY BE EXPOSED TO THIS SUBSTANCE, THE EMPLOYER SHOULD PROVIDE AN EYE WASH FOUNTAIN WITHIN THE IMMEDIATE WORK AREA FOR EMERGENCY USE.

CREATION DATE: 11/08/91 REVISION DATE: 04/29/92

MATERIAL SAFETY DATA SHEET

APPLIED BIOSYSTEMS
850 LINCOLN CENTRE DRIVE
FOSTER CITY, CA 94404
(415) 570-6667 (USA)
0925-825650 (UK)

24 HOUR EMERGENCY RESPONSE
NUMBER: 615-366-2000

SUBSTANCE IDENTIFICATION

CAS NUMBER: 76-05-1
RTECS NUMBER: AJ9625000

SUBSTANCE: TRIFLUOROACETIC ACID

TRADE NAMES/SYNONYMS:
ABI MSDS PART # 901812; P/N 400003; P/N 400137; P/N 400445;
2,2,2-TRIFLUOROACETIC ACID; TRIFLUORACETIC ACID; TRIFLUOROETHANOIC ACID;
PERFLUOROACETIC ACID; ACETIC ACID, TRIFLUORO-;
PROTEIN SEQUENCER REAGENT R3, TFA; PEPTIDE SYNTHESIS REAGENT #2, TFA;
SEPARATION PRODUCTS HPLC GRADE, TFA; UN 2699; C2HF3O2;

CHEMICAL FAMILY:
HALOGEN COMPOUND, ALIPHATIC

MOLECULAR FORMULA:F3-C-C-02-H

MOLECULAR WEIGHT: 114.03

CERCLA RATINGS(SCALE 0-3): HEALTH=3 FIRE=0 REACTIVITY=1 PERSISTENCE=1
NFPA RATINGS(SCALE 0-4): HEALTH=3 FIRE=0 REACTIVITY=1

COMPONENTS AND CONTAMINANTS

COMPONENT: TRIFLUOROACETIC ACID PERCENT: 100.0
CAS# 76-05-1

OTHER CONTAMINANTS: NONE

EXPOSURE LIMITS:
NO OCCUPATIONAL EXPOSURE LIMITS ESTABLISHED BY OSHA, ACGIH, OR NIOSH.

PHYSICAL DATA

DESCRIPTION: COLORLESS, FUMING, HYGROSCOPIC LIQUID WITH A STRONG, PUNGENT ODOR RESEMBLING ACETIC ACID. BOILING POINT: 162 F (72 C)

MELTING POINT: 5 F (-15 C) SPECIFIC GRAVITY: 1.535 PH: STRONGLY ACIDIC

SOLUBILITY IN WATER: VERY SOLUBLE VAPOR DENSITY: 4.0

SOLVENT SOLUBILITY: SOLUBLE IN ALCOHOL, ETHER, ACETONE, CARBON TETRACHLORIDE, HEXANE, BENZENE

FIRE AND EXPLOSION DATA

FIRE AND EXPLOSION HAZARD:
NEGLIGIBLE FIRE HAZARD WHEN EXPOSED TO HEAT OR FLAME.

FIREFIGHTING MEDIA:
DRY CHEMICAL, CARBON DIOXIDE, WATER SPRAY OR REGULAR FOAM
(1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

FOR LARGER FIRES, USE WATER SPRAY, FOG OR REGULAR FOAM
(1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

FIREFIGHTING:
MOVE CONTAINER FROM FIRE AREA IF YOU CAN DO IT WITHOUT RISK. APPLY COOLING WATER TO SIDES OF CONTAINERS THAT ARE EXPOSED TO FLAMES UNTIL WELL AFTER FIRE IS OUT. STAY AWAY FROM ENDS OF TANKS (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5, GUIDE PAGE 60).

EXTINGUISH USING AGENTS INDICATED; DO NOT USE WATER DIRECTLY ON MATERIAL. IF LARGE AMOUNTS OF COMBUSTIBLE MATERIALS ARE INVOLVED, USE WATER SPRAY OR FOG IN FLOODING AMOUNTS. USE WATER SPRAY TO ABSORB CORROSIVE VAPORS. COOL CONTAINERS WITH FLOODING AMOUNTS OF WATER FROM AS FAR A DISTANCE AS POSSIBLE. AVOID BREATHING CORROSIVE VAPORS; KEEP UPWIND.

TOXICITY

TRIFLUOROACETIC ACID:
TOXICITY DATA: 10 GM/M3 INHALATION-RAT LC50; 13500 MG/M3 INHALATION-MOUSE LC50; 1200 MG/KG INTRAVENOUS-MOUSE LD50; 150 MG/KG INTRAPERITONEAL-MOUSE LDLO.
CARCINOGEN STATUS: NONE.
LOCAL EFFECTS: CORROSIVE- INHALATION, SKIN, EYE, INGESTION.
ACUTE TOXICITY LEVEL: TOXIC BY INHALATION.
TARGET EFFECTS: NO DATA AVAILABLE.

HEALTH EFFECTS AND FIRST AID

INHALATION:
TRIFLUOROACETIC ACID:
CORROSIVE/TOXIC.
THE LETHAL DOSE REPORTED IN RATS WAS 10 GM/M3. SEE INFORMATION ON ACIDIC CORROSIVES.

ACIDIC CORROSIVES:
ACUTE EXPOSURE- MAY CAUSE RESPIRATORY TRACT IRRITATION WITH COUGHING, CHOKING, AND POSSIBLY BURNS OF THE MUCOUS MEMBRANES. OTHER INITIAL SYMPTOMS MAY INCLUDE DIZZINESS, HEADACHE, NAUSEA AND WEAKNESS. IN SOME CASES PULMONARY EDEMA MAY DEVELOP, EITHER IMMEDIATELY IN SEVERE CASES, OR MORE LIKELY AFTER A LATENT PERIOD OF 5-72 HOURS. THE SYMPTOMS MAY INCLUDE TIGHTNESS IN THE CHEST, DYSPNEA, FROTHY SPUTUM, AND CYANOSIS. PHYSICAL FINDINGS MAY INCLUDE HYPOTENSION, WEAK, RAPID PULSE AND MOIST RALES. RECOVERY MAY BE PROLONGED AND RELAPSES ARE POSSIBLE. IN SEVERE EXPOSURES, DEATH DUE TO ANOXIA MAY OCCUR WITHIN A FEW HOURS AFTER ONSET OF PULMONARY EDEMA SYMPTOMS OR FOLLOWING A RELAPSE.

CHRONIC EXPOSURE- DEPENDING ON THE CONCENTRATION AND DURATION OF EXPOSURE,

REPEATED OR PROLONGED EXPOSURE MAY CAUSE EROSION OF THE TEETH, INFLAMMATORY AND ULCERATIVE CHANGES IN THE MOUTH, AND POSSIBLY JAW NECROSIS. BRONCHIAL IRRITATION WITH COUGH AND FREQUENT ATTACKS OF BRONCHIAL PNEUMONIA MAY OCCUR. GASTROINTESTINAL DISTURBANCES ARE ALSO POSSIBLE.

FIRST AID- REMOVE FROM EXPOSURE AREA TO FRESH AIR IMMEDIATELY. IF BREATHING HAS STOPPED, GIVE ARTIFICIAL RESPIRATION. MAINTAIN AIRWAY AND BLOOD PRESSURE AND ADMINISTER OXYGEN IF AVAILABLE. KEEP AFFECTED PERSON WARM AND AT REST. TREAT SYMPTOMATICALLY AND SUPPORTIVELY. ADMINISTRATION OF OXYGEN SHOULD BE PERFORMED BY QUALIFIED PERSONNEL. GET MEDICAL ATTENTION IMMEDIATELY.

SKIN CONTACT:
TRIFLUOROACETIC ACID:
CORROSIVE.
VAPORS MAY BE HIGHLY IRRITATING. SEE INFORMATION ON ACIDIC CORROSIVES.

ACIDIC CORROSIVES:
ACUTE EXPOSURE- DIRECT CONTACT MAY CAUSE SEVERE PAIN, BURNS AND POSSIBLY BROWNISH OR YELLOWISH STAINS. BURNS MAY BE DEEP WITH SHARP EDGES AND HEAL SLOWLY WITH SCAR TISSUE FORMATION.
CHRONIC EXPOSURE- EFFECTS DEPEND ON THE CONCENTRATION AND DURATION OF EXPOSURE. REPEATED OR PROLONGED CONTACT MAY RESULT IN DERMATITIS OR EFFECTS SIMILAR TO ACUTE EXPOSURE.

FIRST AID- REMOVE CONTAMINATED CLOTHING AND SHOES IMMEDIATELY. WASH AFFECTED AREA WITH SOAP OR MILD DETERGENT AND LARGE AMOUNTS OF WATER UNTIL NO EVIDENCE OF CHEMICAL REMAINS (AT LEAST 15-20 MINUTES). IN CASE OF CHEMICAL BURNS, COVER AREA WITH STERILE, DRY DRESSING. BANDAGE SECURELY, BUT NOT TOO TIGHTLY. GET MEDICAL ATTENTION IMMEDIATELY.

EYE CONTACT:
TRIFLUOROACETIC ACID:
CORROSIVE.
VAPORS MAY BE HIGHLY IRRITATING. SEE INFORMATION ON ACIDIC CORROSIVES.

ACIDIC CORROSIVES:
ACUTE EXPOSURE- DIRECT CONTACT MAY CAUSE PAIN, LACRIMATION, PHOTOPHOBIA AND BURNS. IN MILD BURNS, THE EPITHELIUM REGENERATES RAPIDLY AND THE EYE RECOVERS COMPLETELY. IN SEVERE CASES, THE EXTENT OF INJURY MAY NOT BE FULLY APPARENT FOR SEVERAL WEEKS. ULTIMATELY, THE WHOLE CORNEA MAY BECOME DEEPLY VASCULARIZED AND OPAQUE RESULTING IN BLINDNESS. IN THE WORST CASES, THE EYE MAY BE TOTALLY DESTROYED.
CHRONIC EXPOSURE- EFFECTS DEPEND ON THE CONCENTRATION AND DURATION OF EXPOSURE. REPEATED OR PROLONGED CONTACT MAY CAUSE CONJUNCTIVITIS OR EFFECTS AS IN ACUTE EXPOSURE.

FIRST AID- WASH EYES IMMEDIATELY WITH LARGE AMOUNTS OF WATER, OCCASIONALLY LIFTING UPPER AND LOWER LIDS, UNTIL NO EVIDENCE OF CHEMICAL REMAINS (AT LEAST 15-20 MINUTES). CONTINUE IRRIGATING WITH NORMAL SALINE UNTIL THE PH HAS RETURNED TO NORMAL (30-60 MINUTES). COVER WITH STERILE BANDAGES. GET MEDICAL ATTENTION IMMEDIATELY.

INGESTION:
TRIFLUOROACETIC ACID:
CORROSIVE.
SEE INFORMATION ON ACIDIC CORROSIVES.

ACIDIC CORROSIVES:
ACUTE EXPOSURE- MAY CAUSE CIRCUMORAL BURNS WITH DISCOLORATION AND CORROSION

OF THE MUCOUS MEMBRANES OF THE MOUTH, THROAT AND ESOPHAGUS. THERE MAY BE IMMEDIATE PAIN AND DIFFICULTY OR INABILITY TO SWALLOW OR SPEAK. EPIGLOTTAL EDEMA MAY RESULT IN RESPIRATORY DISTRESS AND POSSIBLY ASPHYXIA. MARKED THIRST, NAUSEA, VOMITING AND DIARRHEA MAY OCCUR. DEPENDING ON THE AREA AND DEGREE OF CORROSION, THE VOMITUS MAY CONTAIN FRESH OR DARK BLOOD AND LARGE SHREDS OF MUCOSA. SHOCK MAY OCCUR WITH MARKED HYPOTENSION, WEAK AND RAPID PULSE, SHALLOW RESPIRATION, AND CLAMMY SKIN. CIRCULATORY COLLAPSE MAY DEVELOP AND IF UNCORRECTED, LEAD TO RENAL FAILURE. IN SEVERE CASES, GASTRIC AND, TO A LESSER DEGREE, ESOPHAGEAL PERFORATION MAY OCCUR WITH PERITONITIS ACCOMPANIED BY FEVER AND ABDOMINAL RIGIDITY. ESOPHAGEAL, GASTRIC OR PYLORIC STRICTURE MAY OCCUR WITHIN A FEW WEEKS, OR MAY BE DELAYED FOR MONTHS OR EVEN YEARS. DEATH MAY RESULT WITHIN A SHORT TIME FROM ASPHYXIA, CIRCULATORY COLLAPSE OR ASPIRATION OF EVEN MINUTE AMOUNTS. IF DEATH IS DELAYED, IT MAY BE DUE TO PERITONITIS, SEVERE NEPHRITIS OR PNEUMONIA. COMA AND CONVULSIONS SOMETIMES OCCUR TERMINALLY.

CHRONIC EXPOSURE- DEPENDING ON THE CONCENTRATION, REPEATED INGESTION MAY RESULT IN INFLAMMATORY AND ULCERATIVE CHANGES IN THE MUCOUS MEMBRANES OF THE MOUTH AND OTHER EFFECTS AS IN ACUTE INGESTION.

FIRST AID- TREAT SYMPTOMATICALLY AND SUPPORTIVELY. IF PERSON IS CONSCIOUS AND ABLE TO SWALLOW, GIVE LARGE AMOUNTS OF WATER OR MILK TO DILUTE SUBSTANCE. GET MEDICAL ATTENTION IMMEDIATELY. GASTRIC LAVAGE PERFORMED BY QUALIFIED MEDICAL PERSONNEL MIGHT BE ADVISABLE IF THERE ARE NO SIGNS OF PERFORATION FROM THE INGESTION OF A CORROSIVE SUBSTANCE. IF VOMITING OCCURS, KEEP HEAD BELOW HIPS TO HELP PREVENT ASPIRATION.

ANTIDOTE:
NO SPECIFIC ANTIDOTE. TREAT SYMPTOMATICALLY AND SUPPORTIVELY.

REACTIVITY

REACTIVITY:
STABLE UNDER NORMAL TEMPERATURES AND PRESSURES.

INCOMPATIBILITIES:
TRIFLUOROACETIC ACID:
ACIDS: REACTS TO FORM TOXIC GASES.
AROMATIC HYDROCARBONS, HYDROGEN PEROXIDE: EXPLOSION HAZARD.
BASES: REACTS VIOLENTLY.
COMBUSTIBLE MATERIALS: INCOMPATIBLE.
LITHIUM TETRAHYDROALUMINATE: FIRE AND EXPLOSION HAZARD.
METALS: ATTACKS.

DECOMPOSITION:
MAY RELEASE TOXIC AND HAZARDOUS HYDROGEN FLUORIDE FUMES UNDER THERMAL DECOMPOSITION OR BY REACTION WITH ACIDS.

POLYMERIZATION:
HAZARDOUS POLYMERIZATION HAS NOT BEEN REPORTED TO OCCUR UNDER NORMAL TEMPERATURES AND PRESSURES.

STORAGE AND DISPOSAL

OBSERVE ALL FEDERAL, STATE AND LOCAL REGULATIONS WHEN STORING OR DISPOSING OF THIS SUBSTANCE. FOR ASSISTANCE, CONTACT THE DISTRICT DIRECTOR OF THE ENVIRONMENTAL PROTECTION AGENCY.

STORAGE

STORAGE TEMPERATURE: 20 +/- 10 C.
SHELF LIFE: >3 YEARS.

STORE AWAY FROM INCOMPATIBLE SUBSTANCES.

DISPOSAL

DISPOSAL MUST BE IN ACCORDANCE WITH STANDARDS APPLICABLE TO GENERATORS OF HAZARDOUS WASTE, 40 CFR 262. EPA HAZARDOUS WASTE NUMBER D002.
100 POUND CERCLA SECTION 103 REPORTABLE QUANTITY.

CONDITIONS TO AVOID

MAY BURN BUT DOES NOT IGNITE READILY. FLAMMABLE, POISONOUS GASES MAY ACCUMULATE IN TANKS AND HOPPER CARS. MAY IGNITE COMBUSTIBLES (WOOD, PAPER, OIL, ETC.).

SPILL AND LEAK PROCEDURES

OCCUPATIONAL SPILL:
DO NOT TOUCH SPILLED MATERIAL. STOP LEAK IF YOU CAN DO IT WITHOUT RISK. FOR SMALL SPILLS, TAKE UP WITH SAND OR OTHER ABSORBENT MATERIAL AND PLACE INTO CONTAINERS FOR LATER DISPOSAL. FOR SMALL DRY SPILLS, WITH CLEAN SHOVEL PLACE MATERIAL INTO CLEAN, DRY CONTAINER AND COVER. MOVE CONTAINERS FROM SPILL AREA. FOR LARGER SPILLS, DIKE FAR AHEAD OF SPILL FOR LATER DISPOSAL. KEEP UNNECESSARY PEOPLE AWAY. ISOLATE HAZARD AREA AND DENY ENTRY.

PROTECTIVE EQUIPMENT

VENTILATION:
PROVIDE LOCAL EXHAUST OR PROCESS ENCLOSURE VENTILATION SYSTEM.

RESPIRATOR:
THE FOLLOWING RESPIRATORS ARE RECOMMENDED BASED ON INFORMATION FOUND IN THE PHYSICAL DATA, TOXICITY AND HEALTH EFFECTS SECTIONS. THEY ARE RANKED IN ORDER FROM MINIMUM TO MAXIMUM RESPIRATORY PROTECTION.
THE SPECIFIC RESPIRATOR SELECTED MUST BE BASED ON CONTAMINATION LEVELS FOUND IN THE WORK PLACE, MUST BE BASED ON THE SPECIFIC OPERATION, MUST NOT EXCEED THE WORKING LIMITS OF THE RESPIRATOR AND MUST BE JOINTLY APPROVED BY THE NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH AND THE MINE SAFETY AND HEALTH ADMINISTRATION (NIOSH-MSHA).

ANY TYPE 'C' SUPPLIED-AIR RESPIRATOR WITH A FULL FACEPIECE OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE OR WITH A FULL FACEPIECE, HELMET OR HOOD OPERATED IN CONTINUOUS-FLOW MODE.

ANY SELF-CONTAINED BREATHING APPARATUS WITH A FULL FACEPIECE OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE.

FOR FIREFIGHTING AND OTHER IMMEDIATELY DANGEROUS TO LIFE OR HEALTH CONDITIONS:

ANY SELF-CONTAINED BREATHING APPARATUS THAT HAS A FULL FACEPIECE AND IS OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE.

ANY SUPPLIED-AIR RESPIRATOR THAT HAS A FULL FACEPIECE AND IS OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE IN COMBINATION WITH AN AUXILIARY SELF-CONTAINED BREATHING APPARATUS OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE-PRESSURE MODE.

CLOTHING:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE (IMPERVIOUS) CLOTHING AND EQUIPMENT TO PREVENT ANY POSSIBILITY OF SKIN CONTACT WITH THIS SUBSTANCE.

GLOVES:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE GLOVES TO PREVENT CONTACT WITH THIS SUBSTANCE.

EYE PROTECTION:
EMPLOYEE MUST WEAR SPLASH-PROOF OR DUST-RESISTANT SAFETY GOGGLES AND A FACESHIELD TO PREVENT CONTACT WITH THIS SUBSTANCE.

EMERGENCY WASH FACILITIES:
WHERE THERE IS ANY POSSIBILITY THAT AN EMPLOYEE'S EYES AND/OR SKIN MAY BE EXPOSED TO THIS SUBSTANCE, THE EMPLOYER SHOULD PROVIDE AN EYE WASH FOUNTAIN AND QUICK DRENCH SHOWER WITHIN THE IMMEDIATE WORK AREA FOR EMERGENCY USE.

CREATION DATE: 07/31/91 REVISION DATE: July 14, 1993

ABI MODEL 432A *Fast Moc*™ Fmoc CHEMISTRY WASTE PROFILE

EMERGENCY PHONE NUMBERS:

(USA) 415-570-6667, ext. 3333 (UK) 0925-825650

I. Identification

The liquid waste for the 432A *FastMoc* Fmoc Chemistry is collected in a 1-gallon carboy. This waste is a complex mixture of reagents which may have properties of greater hazard than the individual waste components by themselves. **HANDLE THIS MATERIAL WITH EXTREME CAUTION! DO NOT DISPOSE OF THIS WASTE IN SINKS OR DRAINS! THIS MATERIAL SHOULD BE DISPOSED OF AS A REGULATED HAZARDOUS WASTE!**

II. Approximate Composition*

MATERIAL	%	TLV	PEL (Units)	CAS NUMBER
Dimethylformamide (DMF)	88	10ppm	10 ppm	68-12-2
Tetrahydrofuran (THF)	5	200 ppm	200 ppm	109-99-9
Piperidine	5	N/A	N/A	110-89-4
N-Methylpyrolidone (NMP)	0.7	N/A	N/A	872-50-4
Dimethyl sulfoxide (DMSO)	0.7	N/A	N/A	67-68-5
Diisopropylethylamine (DIEA)	0.1	N/A	N/A	7087-68-5
l-Hydroxybenzotriazole (HOBt)	0.1	N/A	N/A	2592-95-2
Tetramethylurea	0.1	N/A	N/A	N/A
Diisopropylethylammonium hexafluoro-phosphate (DIEA - PF_6)	0.1	N/A	N/A	N/A
Amino Acids	0.1	N/A	N/A	N/A

N/A = Not available

III. Physical Data

BOILING POINT 760 mm:	N/A	**FREEZING POINT**	N/A
SPECIFIC GRAVITY (H_20 = 1):	0.94%	**pH RANGE**	N/A
VOLATILITY (vol %)	99%	**SOLUBILITY IN WATER**	Soluble
APPEARANCE AND ODOR	Colorless liquid with a mild, ammonia-like odor.		

IV. Fire and Explosion Hazard Data

FLASH POINT (Closed Cup) THF only:	23 ˚C (74 ˚F)
FLAMMABLE LIMITS (THF only):	1.8 % LEL 11.8 % UEL
FIRE EXTINGUISHING MEDIA	Dry chemical, alcohol foam, carbon dioxide or Halon.

* Percentages of the different waste components will vary depending on the length and sequence of the peptide synthesized, and the version of software used.

SPECIAL FIRE FIGHTING PROCEDURES	Use self-contained breathing apparatus and protective clothing to prevent skin and eye contact.

V. Health Hazard Data

EXPOSURE LIMITS	See Section III. For THF, the STEL is 250 ppm, and the IDLH level is 20,000 ppm.

EFFECTS OF ACUTE EXPOSURE

SWALLOWING	Harmful if swallowed! Causes severe irritation of eyes, nose, and throat. Higher concentrations may cause liver and kidney damage, unconsciousness, and death.
SKIN	May cause severe irritation or burns. Allergic skin sensitization may also occur.
INHALATION	May cause irritation of eyes, nose, throat, and lungs. Higher concentrations may cause pulmonary edema, unconsciousness, and death.

EMERGENCY AND FIRST AID PROCEDURES

SWALLOWING	If conscious, give large volumes of water immediately. Get medical attention immediately.
SKIN	Remove contaminated clothing. Flush the contaminated area with water for at least 15 minutes and wash with mild soap or detergent. Get medical attention.
INHALATION	Provide fresh air and rest. If breathing is difficult, provide oxygen and get medical attention immediately.
EYES	Flush eyes immediately with large amounts of water for at least 15 minutes. Get medical attention.

VI. Reactivity Data

STABILITY:	Stable
INCOMPATIBILITY	Contact with strong oxidizing agents or concentrated acids or bases may cause fire or explosion.
HAZARDOUS COMBUSTION OR DECOMPOSITION	Burning may release toxic vapors and gases, including hydrogen flouride, carbon monoxide, and oxides of nitrogen.
HAZARDOUS POLYMERIZATION:	Will not occur

VII. Spill or Leak Procedures

STEPS TO BE TAKEN

Avoid inhalation and skin contact. Wear protective clothing. Ventilate area of spill or leak. Remove all ignition sources. Small quantities may be collected with absorbant towels or pads and removed to a well-ventilated area away from ignition sources. Larger amounts (1 liter or more) may be collected with an inert absorbant (kitty litter or similar material) or commercially available spill pillows designed for solvent collection. This waste material must not be allowed to enter confined spaces (such as a sewer!!!) because of the possibility of an explosion.

WASTE DISPOSAL METHOD

This instrument waste solution should be disposed of as a regulated hazardous waste by a properly-permitted hazardous waste management facility in accordance with federal, state and local regulations. Recommended disposal methods include high temperature incineration and solidification for secure chemical landfill disposal.

VIII. Special Protective Equipment

RESPIRATORY PROTECTION	An MSHA- or NIOSH- approved respirator for organic vapors is recommended. A supplied-air or SCBA respirator is recommended for high vapor concentration and emergency situations.
VENTILATION	Handle within a well-ventilated area. Minimize open exposure to air.
PROTECTIVE GLOVES	Neoprene or latex rubber gloves are recommended.
EYE PROTECTION	Safety glasses with side shields, monogoggles or face shield**.**
OTHER PROTECTIVE EQUIPMENT	As necessary, to prevent skin contact.

IX. Special Precautions

PRECAUTIONS TO BE TAKEN

Handle as a flammable, poisonous liquid. Maintain adequate ventilation at all times. Do not breathe vapor. Do not get in eyes, on skin, or on clothing. Accidental contact should be washed immediately. Keep away from heat, sparks, and flame. Keep containers tightly closed. Spill collection materials, eye wash, and safety shower should be in area of use.

OTHER

This waste solution has strong solvent properties and will attack many forms of rubber, plastics, coating, fabrics, and finishes.

X. Additional Information

When not directly attached to the instrument, this waste material should be stored in a secure, well-ventilated location suitable for flammable materials. Store away from light, heat, or potential ignition sources. Contact the appropriate state hazardous waste regulatory agency for proper disposal procedures and list of registered service companies.

THIS WASTE MATERIAL IS HAZARDOUS AND SHOULD ONLY BE HANDLED BY PERSONS THOROUGHLY TRAINED IN HAZARDOUS MATERIALS HANDLING PROCEDURES!

DATE ISSUED: July 14, 1993

Appendix II AB Limited Warranty

Applied Biosystems (“AB”) warrants to the customer that, for a period ending on the earlier of one year from completion of installation or 13 months from the date of shipment to the customer (the “Warranty Period”), the AB Model 432A (the “Instrument”) purchased by the customer will be free from defects in material and workmanship, and will perform at least in accordance with the minimum performance specifications for flow test (the “Mechanical Performance”) and synthesis of the test peptide, LAGV, (the “Chemical Performance”) as specified in the Install Guide.

This Warranty does not extend to any Instrument or part thereof that has been subjected to misuse, neglect or accident, that has been modified or repaired by anyone other than AB or that has not been used in accordance with the instructions contained in the Instrument User's Manual. Nor does this Warranty cover any customer-installable consumable parts for the Instrument that are listed in the Instrument User's Manual.

This Warranty does not apply to the Instrument's valves or reagent lines, unless the customer uses only reagents and solvents supplied by AB, or those of highest purity available that have been filtered to be free of particulates, or those proven to be completely dissolved using AB cycles. If the use of reagents or solvents supplied by AB, or those of highest purity available that have been filtered to be free of particulates, or those proven to be completely dissolved using AB cycles causes such hardware damage or contamination during the Warranty Period, AB will return the Instrument to specified Mechanical Performance at AB's expense. However, AB does not guarantee specified Chemical Performance of the Instrument if solvents or reagents other than those supplied by AB are used. If the Chemical Performance of the Instrument deteriorates due to solvents and/or reagents other than those supplied by AB, at the customer's request AB will return the Instrument to specified Chemical Performance and Mechanical Performance at the customer's expense. If only reagents and solvents supplied by AB are used thereafter, the Chemical Performance and Mechanical Performance will be covered in full for the remainder of the Warranty Period.

AB's obligation under this Warranty is limited to repairs or replacements that AB deems necessary to correct covered defects or failures of which AB is notified prior to expiration of the Warranty Period. All repairs and replacements determined to be covered by this Warranty shall be performed by AB.

No agent, employee, or representative of AB, other than an officer of the company, has any authority to bind AB to any affirmation, representation, or Warranty concerning the Instrument that is not contained in the User's Manual or this Warranty.

AB shall not be liable for any incidental, special, or consequential loss, damage or expense directly or indirectly arising from the purchase or use of the Instrument. AB makes no Warranty whatsoever in regard to products or parts furnished by third parties.

This Warranty is limited to the original customer and is not transferable.

THIS WARRANTY IS THE SOLE AND EXCLUSIVE WARRANTY AS TO THE INSTRUMENT AND IS EXPRESSLY IN LIEU OF ANY OTHER EXPRESS OR IMPLIED WARRANTIES, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABLITY OR FITNESS FOR A PARTICULAR PURPOSE KNOWN TO THE SELLER AND OF ANY OTHER OBLIGATION ON THE PART OF APPLIED BIOSYSTEMS.

Appendix III Synergy Parts and Reagents

The following listed parts for the Synergy Personal Peptide Synthesizer may be ordered by Synergy users and replaced without removing the instrument's exterior panels.

User-Replaceable Synergy Parts

Part Description	Part Number
amino acid column wheel	004185
calibration test fixtures	401383
waste cap assembly	602851
waste port nut, ferrule, and gripper	230034
bottle cap assembly (1 gal bottle)	601396
bottle seals—40 mL bottles (10/pkg.)	400612
bottle seals—200 mL bottles (10/pkg.)	400790
safety carrier (1 gal bottle)	140041
RS232 printer cable	100390

Synergy Synthesis Reagents by Bottle Position

Bottle Position	Reagent Description	Quantity	Part Number
A	HBTU	8 mmol	401278
	HOBt/DMSO/NMP	40 mL	401279
B	DIEA/DMSO/NMP	40 mL	401254
1	Piperidine	200 mL	400629
2	THF, stabilized	200 mL	401255
10	DMF	4 L	400143

The Synergy 100 Cycle Reagent Kit, P/N 401466, contains enough liquid reagents for approximately 100 synthesis cycles and includes: two 200-mL bottles piperidine, two 200-mL bottles THF, two 4-L bottles DMF, one 8-mmol bottle HBTU, one 40-mL bottle HOBt/DMSO/NMP, and one 40-mL bottle DIEA/DMSO/NMP.

Amino acid columns* (AACs)

Amino Acid	Part Number
Alanine/Ala	401208
Arginine/Arg (Pmc)	401209
Asparagine/Asn (Trt)	401210
Aspartate/Asp (OtBu)	401211
Cysteine/Cys (Trt)	401212
Glutamine/Gln (Trt)	401213
Glutamate/Glu (OtBu)	401214
Glycine/Gly	401215
Histidine/His (Trt)	401216
Isoleucine/Ile	401217
Leucine/Leu	401218
Lysine/Lys (Boc)	401219
Methionine/Met	401220
Phenylalanine/Phe	401221
Proline/Pro	401222
Serine/Ser (tBu)	401223
Threonine/Thr (tBu)	401224
Tryptophan/Trp	401225
Tyrosine/Tyr (tBu)	401226
Valine/Val	401227

*Each amino acid column contains 75 µmol amino acid

Peptide Synthesis Columns* (PSCs)

Amino Acid	Part Number
Alanine/Ala	401228
Arginine/Arg (Pmc)	401229
Asparagine/Asn (Trt)	401230
Aspartate/Asp (OtBu)	401231
Cysteine/Cys (Trt)	401232
Glutamine/Gln (Trt)	401233
Glutamate/Glu (OtBu)	401234
Glycine/Gly	401235
Histidine/His (Trt)	401236
Isoleucine/Ile	401237
Leucine/Leu	401238
Lysine/Lys (Boc)	401239
Methionine/Met	401240
Phenylalanine/Phe	401241
Proline/Pro	401242
Serine/Ser (tBu)	401243
Threonine/Thr (tBu)	401244
Tryptophan/Trp	401245
Tyrosine/Tyr (tBu)	401246
Valine/Val	401247
Amide	401248

*Each PSC contains 25 µmol of pre-loaded or amide resin

Fmoc MAP Resin Columns (PSCs)

Fmoc MAP resin	Part Number
4-branched MAP	401391
8-branched MAP	401392

Appendix IV Test Printouts

This appendix contains printouts of all the steps and functions that compose each of the flow tests and the Vent Test. The Vent Test is performed only during Synergy Installation, while the waste port is plugged, to check for internal leaks in the ventilation system.

Contents

Flow Test 1: DMF->L/R/PSC

Step#	Function#	Function Name	Time
1	3	BEGIN LOOP	999
2	84	PRS PSC	10
3	88	+PRS TEST	30
4	89	-PRS TEST	1
5	4	END LOOP	1
6	68	L>WASTE	3
7	61	XFER	3
8	68	L>WASTE	3
9	2	INTERRUPT	1
10	30	DMF>TVB	3
11	1	WAIT	10
12	35	DMF>L	3
13	10	GAS>TVB	3
14	14	GAS>L	1
15	2	INTERRUPT	2
16	68	L>WASTE	3
17	61	XFER	3
18	68	L>WASTE	3
19	31	DMF>BVB	3
20	1	WAIT	10
21	36	DMF>R	3
22	11	GAS>BVB	3
23	15	GAS>R	1
24	61	XFER	3
25	15	GAS>R	1
26	61	XFER	3
27	2	INTERRUPT	3
28	68	L>WASTE	3
29	31	DMF>BVB	3
30	34	DMF>PSC(B)	30
31	68	L>WASTE	3
32	61	XFER	3
33	68	L>WASTE	3
34	30	DMF>TVB	3
35	1	WAIT	30
36	39	DMF>PSC>R	20
37	11	GAS>BVB	3
38	15	GAS>R	1
39	61	XFER	3
40	15	GAS>R	1
41	61	XFER	3
42	2	INTERRUPT	4
43	68	L>WASTE	3
44	61	XFER	3
45	68	L>WASTE	3
46	10	GAS>TVB	3
47	13	GAS>PSC	30
48	11	GAS>BVB	3

Flow Test 2: CALIBRATE COLUMN

Step#	Function#	Function Name	Time
1	3	BEGIN LOOP	999
2	84	PRS PSC	10
3	88	+PRS TEST	30
4	89	-PRS TEST	1
5	4	END LOOP	1
6	31	DMF>BVB	3
7	34	DMF>PSC(B)	5
8	10	GAS>TVB	3
9	13	GAS>PSC	30
10	31	DMF>BVB	3
11	1	WAIT	30
12	34	DMF>PSC(B)	9
13	2	INTERRUPT	1
14	34	DMF>PSC(B)	2
15	2	INTERRUPT	2
16	10	GAS>TVB	3
17	13	GAS>PSC	30
18	23	THF>PSC	10
19	10	GAS>TVB	3
20	13	GAS>PSC	30
21	10	GAS>TVB	3
22	11	GAS>BVB	3

Flow Test 3: DMF -> PSC

Step#	Function#	Function Name	Time
1	3	BEGIN LOOP	999
2	84	PRS PSC	10
3	88	+PRS TEST	30
4	89	-PRS TEST	1
5	4	END LOOP	1
6	31	DMF>BVB	3
7	34	DMF>PSC(B)	5
8	10	GAS>TVB	3
9	13	GAS>PSC	30
10	31	DMF>BVB	3
11	1	WAIT	30
12	34	DMF>PSC(B)	10
13	2	INTERRUPT	1
14	10	GAS>TVB	3
15	13	GAS>PSC	30
16	23	THF>PSC	10
17	10	GAS>TVB	3
18	13	GAS>PSC	30
19	10	GAS>TVB	3
20	11	GAS>BVB	3

Flow Test 4: THF ->PSC

Step#	Function#	FunctionName	Time
1	3	BEGIN LOOP	999
2	84	PRS PSC	10
3	88	+PRS TEST	30
4	89	-PRS TEST	1
5	4	END LOOP	1
6	21	THF>BVB	3
7	24	THF>PSC(B)	5
8	10	GAS>TVB	3
9	13	GAS>PSC	30
10	21	THF>BVB	3
11	1	WAIT	30
12	24	THF>PSC(B)	7
13	2	INTERRUPT	1
14	10	GAS>TVB	3
15	13	GAS>PSC	30
16	10	GAS>TVB	3
17	11	GAS>BVB	3

Flow Test 5: PIP-> PSC

Step#	Function#	Function Name	Time
1	3	BEGIN LOOP	999
2	84	PRS PSC	10
3	88	+PRS TEST	30
4	89	-PRS TEST	1
5	4	END LOOP	1
6	86	+PRS PIP	30
7	44	PIP>WASTE	3
8	43	PIP>PSC(B)	5
9	10	GAS>TVB	3
10	13	GAS>PSC	30
11	44	PIP>WASTE	3
12	1	WAIT	30
13	43	PIP>PSC(B)	22
14	2	INTERRUPT	1
15	87	-PRS PIP	1
16	10	GAS>TVB	3
17	13	GAS>PSC	30
18	3	BEGIN LOOP	3
19	30	DMF>TVB	3
20	33	DMF>PSC	10
21	10	GAS>TVB	3
22	13	GAS>PSC	20
23	4	END LOOP	1
24	23	THF>PSC	10
25	10	GAS>TVB	3
26	13	GAS>PSC	30
27	10	GAS>TVB	3
28	31	DMF>BVB	3
29	11	GAS>BVB	3

Flow Test 6: PUMP/AAC

Step#	Function#	Function Name	Time
1	53	OPEN JAWS	5
2	51	HOME AAC	5
3	52	CLOSE JAWS	5
4	3	BEGIN LOOP	999
5	83	PRS AAC	10
6	88	+PRS TEST	30
7	89	-PRS TEST	1
8	4	END LOOP	1
9	15	GAS>R	3
10	61	XFER	3
11	68	L>WASTE	3
12	30	DMF>TVB	3
13	35	DMF>L	4
14	2	INTERRUPT	1
15	62	+AAC PATH	1
16	60	PUMP	7
17	63	-AAC PATH	1
18	2	INTERRUPT	2
19	61	XFER	2
20	2	INTERRUPT	3
21	68	L>WASTE	6
22	22	THF>AAC	3
23	12	GAS>AAC	60
24	15	GAS>R	3
25	61	XFER	3
26	68	L>WASTE	3
27	53	OPEN JAWS	5

Flow Test7: PUMP/CELL

Step#	Function#	Function Name	Time
1	3	BEGIN LOOP	999
2	84	PRS PSC	10
3	88	+PRS TEST	30
4	89	-PRS TEST	1
5	4	END LOOP	1
6	31	DMF>BVB	3
7	34	DMF>PSC(B)	30
8	2	INTERRUPT	1
9	30	DMF>TVB	3
10	35	DMF>L	4
11	45	HBTU>L	2
12	14	GAS>L	5
13	30	DMF>TVB	3
14	35	DMF>L	1
15	64	+PSC PATH	1
16	3	BEGIN LOOP	5
17	60	PUMP	14
18	61	XFER	1
19	4	END LOOP	1
20	65	-PSC PATH	1
21	2	INTERRUPT	2
22	68	L>WASTE	6
23	3	BEGIN LOOP	3
24	36	DMF>R	3
25	15	GAS>R	3
26	61	XFER	3
27	68	L>WASTE	3
28	4	END LOOP	1
29	61	XFER	3
30	68	L>WASTE	3
31	30	DMF>TVB	3
32	33	DMF>PSC	60
33	13	GAS>PSC	30
34	20	THF>TVB	3
35	23	THF>PSC	30
36	10	GAS>TVB	3
37	13	GAS>PSC	60
38	11	GAS>BVB	3

Flow Test 8: CALIBRATE HBTU

Step#	Function#	Function Name	Time
1	10	GAS>TVB	5
2	3	BEGIN LOOP	2
3	15	GAS>R	3
4	61	XFER	3
5	68	L>WASTE	3
6	4	END LOOP	1
7	2	INTERRUPT	1
8	10	GAS>TVB	5
9	45	HBTU>L	10
10	14	GAS>L	5
11	2	INTERRUPT	2
12	45	HBTU>L	10
13	14	GAS>L	5
14	2	INTERRUPT	3
15	68	L>WASTE	6
16	3	BEGIN LOOP	2
17	15	GAS>R	3
18	61	XFER	3
19	68	L>WASTE	3
20	4	END LOOP	1
21	2	INTERRUPT	4
22	10	GAS>TVB	5
23	45	HBTU>L	14*
24	14	GAS>L	5
25	2	INTERRUPT	5
26	68	L>WASTE	6
27	36	DMF>R	2
28	15	GAS>R	2
29	61	XFER	3
30	68	L>WASTE	3
31	3	BEGIN LOOP	2
32	15	GAS>R	3
33	61	XFER	3
34	68	L>WASTE	3
35	4	END LOOP	1

* Substitute time.

Flow Test 9: CALIBRATE DIEA

Step#	Function#	Function Name	Time
1	10	GAS>TVB	5
2	3	BEGIN LOOP	2
3	15	GAS>R	3
4	61	XFER	3
5	68	L>WASTE	3
6	4	END LOOP	1
7	2	INTERRUPT	1
8	10	GAS>TVB	5
9	46	DIEA>L	10
10	14	GAS>L	5
11	2	INTERRUPT	2
12	46	DIEA>L	10
13	14	GAS>L	5
14	2	INTERRUPT	3
15	68	L>WASTE	6
16	3	BEGIN LOOP	2
17	15	GAS>R	3
18	61	XFER	3
19	68	L>WASTE	3
20	4	END LOOP	1
21	2	INTERRUPT	4
22	10	GAS>TVB	5
23	46	DIEA>L	11*
24	14	GAS>L	5
25	2	INTERRUPT	5
26	68	L>WASTE	6
27	36	DMF>R	2
28	15	GAS>R	2
29	61	XFER	3
30	68	L>WASTE	3
31	3	BEGIN LOOP	2
32	15	GAS>R	3
33	61	XFER	3
34	68	L>WASTE	3
35	4	END LOOP	1

* Substitute time.

VENT TEST

Step#	Function#	Function Name	Time
1	2	INTERRUPT	1
2	88	+PRS TEST	30
3	89	-PRS TEST	10
4	2	INTERRUPT	2
5	10	GAS>TVB	10
6	88	+PRS TEST	30
7	89	-PRS TEST	10
8	14	GAS>L	10
9	88	+PRS TEST	30
10	89	-PRS TEST	10
11	15	GAS>R	10
12	88	+PRS TEST	30
13	89	-PRS TEST	10
14	2	INTERRUPT	3
15	55	+FUNCTION	10
16	57	+VALVE	18
17	1	WAIT	60
18	2	INTERRUPT	4
19	58	-VALVE	18
20	56	-FUNCTION	10

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