FAMOS™

Well Plate Microautosampler

User's Manual

P/N 160557

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D931R1

Notice: The FAMOS™ Well Plate Microautosampler is covered by a limited warranty. A copy of this warranty is included with this manual. The customer is required to perform routine maintenance as described in the User's Manual on a periodic basis to keep the warranty in effect.

All information in this manual is subject to change without notice and does not represent a commitment on the part of LC Packings, BV.

The material included in this manual is provided to assist users in the operation, maintenance and repair of the FAMOS Well Plate Microautosampler. It is assumed that the individual using this manual has sufficient training in the use of analytical instrumentation and is aware of the potential hazards including (but not limited to) electrical hazards, chemical solvent hazards and the exposure to pressurized solvents.

Printed in the Netherlands.

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Warranty

LC Packings (Netherlands) BV, warrants that the products manufactured and sold by it to be free from defects in material and workmanship for normal use and service from the date of delivery to original purchaser for a period of one (1) year from the date of shipment. This limited warranty does not cover, and no warranty is provided, for parts that by their nature are required to be replaced periodically as a function of use of the normal operation of the system. These items include, without limitation: HPLC columns, fuses, tubing, detector sources, pump piston seals, injector rotors, check valves, filters, any software, etc. In addition, damage due to corrosion, misuse, negligence, accident, alteration of the system or repair by an unauthorized individual is not covered by the warranty. It is understood that the performance characteristics of the instrument require that the mobile phase be degassed with He as described in the User's Manual.

This warranty covers products sold under the LC Products trademark. If a different warranty than the above is indicated in the sales literature, the warranty indicated in the sales literature will prevail. If the system includes equipment supplied by LC Packings but manufactured by a third party, LC Packings makes no warranty of any kind, express or implied, including, without limitation, any warranty of merchantability or fitness for a particular purpose. LC Packings will make available to you, to the extent permitted, the warranties of the manufacturer of the relevant equipment following your timely written request.

If any product covered by this warranty becomes defective during the warranty period, it will be repaired or replaced by LC Packings at no charge to the customer (the repair/replace decision is solely at the option of LC Packings). All warranty requests must be received by LC Packings during the warranty period.

LC Packings will pay for surface transportation to the applicable LC Packings Office (North America - San Francisco CA, Europe and Asia - Amsterdam, the Netherlands), if the instrument proves defective within thirty (30) days from the date of shipment (this does not include air freight, drayage, labor, crating charges, customs clearance charges, etc.). The user should carefully follow the directions indicated on the Return Goods Instruction Sheet in the User's Manual. After thirty days, all transportation costs will be at the expense of the customer.

Software Warranty

If, at any time during the period ending ninety (90) days after delivery of any product to you, you report and document any error in any software provided with such product and developed by LC Packings or any failure of any such software substantially to conform to LC Packings software description that limits or prevents use of the software by you, we will use reasonable efforts to correct any such error or failure, will replace such software or will terminate your license to use the software and refund the price of the related product. In connection with any such termination and refund, you will return the related product to LC Packings upon request.

The warranty will apply only to those portions of the software that were developed by LC Packings and that incorporated all program corrections and modifications, if any, delivered to you. It will not apply to any error or failure due to machine error or to the misuse by or negligence of any person or entity other than LC Packings or to any software, which is modified by any person, or entity other than LC Packings.

Liability

Under no circumstances shall LC Packings be liable for damage to persons or property. This warranty is the only warranty given by LC Packings with respect to products and software provided with the products and is given in lieu of all other warranties, express or implied, including, without limitation, any warranty of merchantability or fitness for a particular purpose.

Your exclusive remedies and LC Packings's sole liability for any non-conformity or defect in the products and such software will be those expressed herein. Under no circumstances will LC Packings's liability arising from the performance or failure to perform of any product or software, in contract, in tort (including negligence), or otherwise, exceed the purchase price of the product and software. In no event will LC Packings be liable, in contract, in tort (including negligence), or otherwise for special, incidental, consequential or analogous damages, including, without limitation, damages resulting from loss of use, loss of profits, loss of business or loss of goodwill, even if LC Packings has been advised of the possibility of such damages.

This warranty comprises the entire warranty between LC Packings and the customer. It overrides any warranty related language that may appear in the customer purchase order or other documentation provided by the customer.

This warranty shall be governed by, and construed and enforced in accordance with, the laws of the Netherlands. It is non-transferable and shall run to the benefit of the original purchaser only. Any change, alteration or amendment to this warranty is not valid unless it has been approved in writing by an officer of LC Packings.

Warrantor North America
LC Packings (U.S.A.) Inc.
80 Carolina Street
San Francisco CA 94103
USA

Phone: (415) 552-1855
Fax: (415) 552-1858

Warrantor Europe and Asia
LC Packings (Netherlands) BV
Abberdaan 114
1046 AA Amsterdam
The Netherlands

Phone: + 31 20 683 9768
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Instructions for Returning Instruments

Before you return any item for repair, please contact the nearest LC Packings office or its local distributor for instructions and obtain a return authorization number.

Pack the equipment carefully, preferably in its original carton and ship it to the LC Packings Service Department, using the appropriate address.

North America
LC Packings (U.S.A.) Inc.
80 Carolina Street
San Francisco CA 94103
USA

Phone: (415) 552-1855
Fax: (415) 552-1858

Europe and Asia
LC Packings (Netherlands) BV
Abberdaan 114
1046 AA Amsterdam
The Netherlands

Phone: + 31 20 683 9768
Fax: + 31 20 685 3452

IMPORTANT:

1) Make certain that the return authorization number is indicated on the address label of the package so that we can properly track and account for your system.

2) Please include the following

    a) Company letterhead with the following information.

        - Your Name
        - Complete Mailing Address
        - Telephone Number, fax number and e-mail address
        - Return Authorization Number
        - A detailed description of the problem.
        - The name of the LC Packings personnel to whom you have spoken to regarding the problem
        - Return Shipping Information (if appropriate)

    b) Relevant chromatograms

    c) A purchase order (if the system is not in warranty)

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Warnings

The Danger sign, Warning sign and the Hazard sign shown below are included in various locations in this manual. These signs provide the following information:

Danger: The information in a danger statement relates to a procedure, practice condition or action that if not done correctly or adhered to could lead to personal injury or loss of life.

Warning: The information in a warning statement relates to a procedure, practice condition or action that if not done correctly or adhered to could lead to severe injury and/or damage or destruction to parts or all of the equipment.

Caution: The information in a caution statement relates to a condition that could lead to damage to equipment and/or lead to invalid analytical results.

Note: The information in a note statement relates to important information that should be read and understood before continuing.

Safety Precautions

Note: The following precautions should be followed to minimize the possibility of personal injury and/or damage to property.

Note: Make certain that you are familiar with the contents of this manual before working on the system.

1) The system should be installed in a well-ventilated laboratory. If the mobile phase includes volatile or flammable solvents, make certain that they are not allowed to enter the workspace.

2) If the mobile phase includes volatile or flammable solvents, avoid open flames and sparks.

3) If a leak occurs, turn off power to the instrument and remedy the situation immediately.

4) All components of the system should be plugged into a common power line that is directly connected to a true ground.

5) When the panels are removed dangerous electrical connections will be exposed. Disconnect the autosampler from all power sources before removing the panels.

6) Always replace blown fuses with fuses of the same size and rating indicated on the fuse holder and panel. Refer to Section 6.3.6 of this manual for more information on Fuses

7) Repair or replace faulty power cords and all communication cables.

8) Many organic solvents and buffers are toxic. Make certain that you know the toxicological properties of all mobile phases that you are using.

9) The toxicological properties of many samples may not be well known. If you have any doubt about a sample, treat it as if it contained a potentially harmful substance.

10) Wear protective eye goggles when handling mobile phases or operating the instrument. An eye wash facility and a sink should be close to the unit. If any mobile phase splash on the eyes or skin, wash the affected area and seek medical attention.

11) Dispose of all waste mobile phase in an environmentally safe manner that is consistent with all local regulations. Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose of flammable and/or toxic solvents through the municipal sewage system

12) PEEK tubing is used in a variety of locations. While this polymer has superb chemical resistance to most organic solvents, it tends to swell when it is contact with $CHCl_3$, DMSO and THF. In addition, it is attacked by concentrated acids such as Sulfuric Acid and Nitric Acid (swelling or attack by acid is not a problem with short flushing procedures).

    Do not use PEEK tubing that is stressed, bent or has a kink.

13) Wear protective eye goggles when handling fused silica tubing (i.e. installation, cutting etc.)

14) If a buffer is used as a part of the mobile phase, flush the system with several volumes of a methanol/water (50/50) before it is shut down. This will prevent salt buildup inside the unit.

15) Do not use the FAMOS Well Plate Microautosampler in ways other than those indicated in the instructions given in this manual.

16) The following symbols are used on the FAMOS Well Plate Microautosampler:

This indicates that care should be taken to prevent personal injury or damage to parts of the FAMOS Well Plate.

This sticker (with yellow background color) at the back of the FAMOS Well Plate Microautosampler calls attention to the fact that you are expected to consult this manual for instructions on how to operate the FAMOS Well Plate Microautosampler

CE

DECLARATION OF CONFORMITY

We

LC Packings Nederland BV
A Dionex Company
Abberdaan 114
1046 AA Amsterdam
The Netherlands

declare that our product

FAMOS™ Well Plate Microautosampler

is in confirmation with the following documents:

EEC directives 89/392, incl. 91/368 and 93/44 (machine safety) and EEC directives 73/23 and 93/68 (low voltage safety), applied with the following standard:

EN61010-1	Safety requirements for laboratory equipment (Class I, Installation cat. II, Pollution degree II)

WARNING

LC Packings will not accept any liability for damages direct or indirect caused by connecting this instrument to devices which do not meet relevant safety standards.

EEC directives 89/336 and 92/31 (EMC requirements, applied with the following standards:

EN 55011	Radio frequency emission
EN 50082-1	Voltage fluctuations
EN 61000-3-2	Harmonic current emissions

Use shielded cables and connectors for all remote connections.

Amsterdam, January 11, 2001

Robert van Ling, QA manager

D932R1

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CHAPTER 1

System Overview

1.1 Features and Design of the FAMOS™ Well Plate Microautosampler

The FAMOS™ Well Plate Microautosampler is a flexible and powerful system for micro-HPLC and incorporates the following features:

- It can be used with a broad range of plates from various manufacturers (96 well plate, 96 deep well plate, 384 well plate, 48 1.5 mL autosampler vials, sealed or open).
- It is capable of executing full loop injections, partial loopfill injections and µL pickup.
- It supports "Low Dispersion" injection control to optimize injection profiles.
- It can use a broad range of syringes (volumes of 25, 100, 250, 500 or 1000 µL).
- It is fully controlled by *UltiChrom*™ software.
- It is fully compatible with other laboratory equipment.
- It can be used for both routine analysis and method development purposes.
- It is menu driven and includes a broad variety of sample-handling programs such as sampling, diluting, mixing and precolumn derivatization.

A number of options (e.g. Peltier plate cooling and a column oven) are available for the FAMOS Well Plate Microautosampler. In addition, a broad range of components to configure the unit to meet your requirements (e.g. loops, injection needles, syringes, needle holder for nanospray) and disposable items (e.g. vials and caps) are available. A detailed list of available items is presented in Section 6.6.

1.2 Principle of Operation of the Microautosampler

A schematic diagram of the FAMOS Well Plate Microautosampler is presented in FIGURE 1-1.

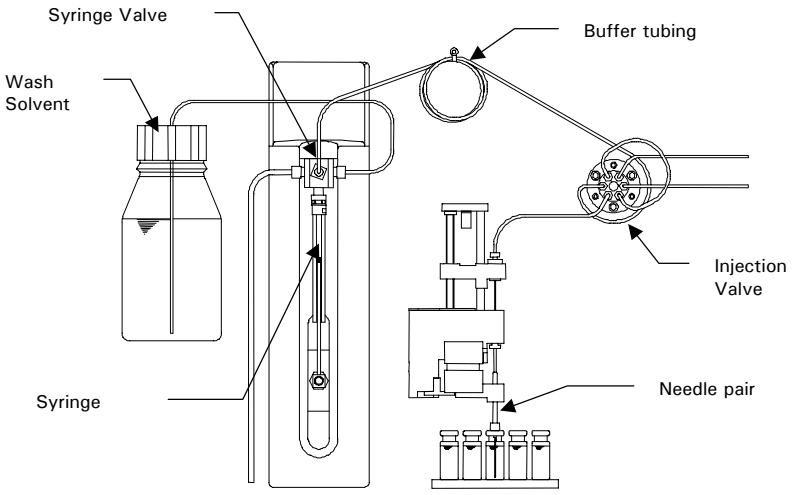


FIGURE 1-1. Schematic Diagram of the FAMOS Well Plate Microautosampler

The FAMOS Well Plate Microautosampler includes the following components:

- **Injection Valve** - includes the loop to place the sample in the mobile phase.
- **Needle Pair** - used to pressurize the sample vial and withdraw sample.
- **Buffer Tubing** - provided to eliminate contamination of the syringe (especially useful with small samples).
- **Syringe** - used to aspirate the sample from a well into the sample loop. To prevent contamination of the syringe, the autosampler is equipped with buffer tubing between the syringe and the injection valve.
- **Wash solvent** is used to remove the sample from the buffer tubing and sample needle, and to rinse the buffer tubing and sample needle.

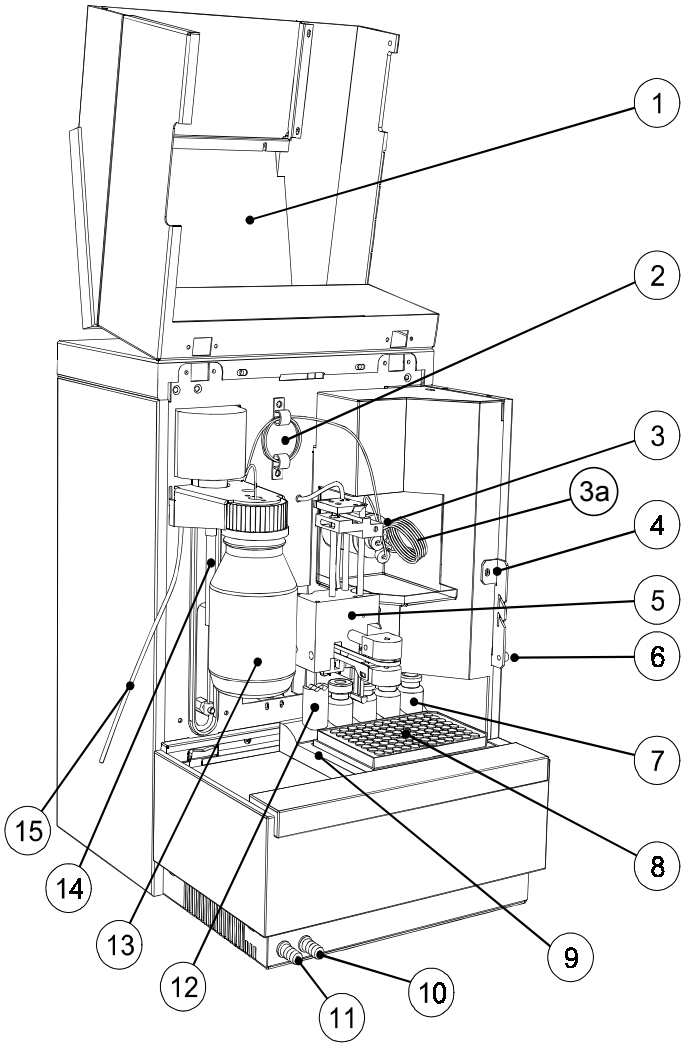
The FAMOS Well Plate Microautosampler provides three different methods of injection for an analytical run:

- **Full loop** - The sample loop is completely (quantitatively) filled with sample. This results in extremely good reproducibility.
- **Partial loopfill** - The sample loop is partially filled with sample. This provides low sample loss. The operator can select the desired injection volume.
- ***µ*L pick-up** - After aspiration of sample, the sample is transported into the loop with transport liquid (mobile phase). In this mode, no sample is lost.

Technical information on the injection principle used by the FAMOS Well Plate Microautosampler is presented in Appendix A.

1.3 General Design of the FAMOS Well Plate Microautosampler

The front view of the FAMOS Well Plate Microautosampler is presented in FIGURE 1-2.



1 Cover (in open position)
2 Buffer tubing
3 Injection valve including the Sample Loop (3a)
4 Tubing holder
5 Needle unit
6 Screw to fasten cover
7 Transport/reagent vials
8 Plate
9 Plate holder
10 Drain wash-position
11 Condensed water and leakage
12 Wash position
13 Wash solvent bottle
14 Syringe
15 Syringe waste tubing

FIGURE 1-2. Front View of the FAMOS Well Plate Microautosampler

1.4 Contents of this Manual

Note: This manual covers the standard version of the FAMOS Well Plate Microautosampler as well as the inert version. If you are using an inert version, please refer to Appendix G, which includes specific information that relates to this configuration and the appropriate part numbers for replacement parts.

This manual describes the FAMOS Well Plate Microautosampler and includes the following information:

Chapter 2: *Installation and Getting Started* describes how to install the FAMOS Well Plate Microautosampler.

Chapter 3: *The User Interface* describes the use of the display panel and the user interaction program to establish methods and series

Chapter 4: *Programming Examples* presents details of a variety of methods/series for that represent common applications of the microautosampler.

Chapter 5: *Testing the Microautosampler* includes a protocol that can be used to verify that the Microautosampler is operating in an acceptable manner.

Chapter 6: *Maintenance and Troubleshooting* describes a variety of maintenance procedures to optimize the performance of the microautosampler. In addition, it discusses how the operator can determine the cause of a difficulty in the operation of the autosampler and includes a list of spare/replacement parts

Chapter 7: *Specifications* presents the specifications of the FAMOS Well Plate Microautosampler

In addition, a series of appendices are provided to supply information about the injection principles, a detailed chart that presents the operation program, additional programming examples, a discussion about the injection valve and the various inputs and outputs on the rear panel.

If you are using the FAMOS Well Plate Microautosampler with the LC Packings *UltiMate*™ and/or *UltiChrom* software, please refer to the documentation provided with these products for supplemental information. If the microautosampler is used with other systems, the manuals provided with these systems should be consulted for interfacing requirements.

CHAPTER 2

Installation and Getting Started

2.1 Installation

The instructions provided below are provided for installation of the FAMOS™ Well Plate Microautosampler as a stand-alone component in an HPLC system. When the FAMOS Well Plate Microautosampler is used in conjunction with a LC Packings *UltiMate*™ system, please refer to the *UltiMate* User's Manual for additional information.

Once you have set up the microautosampler, refer to Section 2.6 for information about routine operation of the system.

2.1.1 Location of the FAMOS Well Plate Microautosampler in the Laboratory

The FAMOS Well Plate Microautosampler should be installed in a facility with the following environmental conditions:

- The temperature range should be maintained between 10 and 40°C. The system should be installed in an area in which the temperature is fairly constant (do not place the system near a window, an air conditioning duct or a heating duct). The humidity should be maintained between 20 and 80 % relative humidity.
- If flammable or toxic solvents are to be used, a suitable ventilation system should be provided.
- The use of open flames in the laboratory should be prohibited.
- Corrosive vapors or dust should not be present as these materials can adversely affect the long-term performance of the system.

The microautosampler requires approximately 280 mm (11.2") of linear bench space. The lab bench should be capable of supporting 100 kg (225 lb.).

The power consumption of the FAMOS Well Plate Microautosampler is 250 VA.

Danger: The FAMOS Well Plate Microautosampler must be connected to a power source that is connected to a true ground. In addition, all other components of the system (e.g. the HPLC pump, the detector) should be connected to the same ground.

Caution: Do not install the FAMOS Well Plate Microautosampler in areas subject to shock, dust, or in direct sunlight. Do not place it near a source of heat (this is especially important if the tray cooling option is installed.

2.2 Unpacking

When the FAMOS Well Plate Microautosampler is received, carefully unpack the unit and verify receipt of all components according to the packing list (some components include sub-packing lists). It is recommended that all packing materials be saved in the event that it is necessary to return any item to the factory.

Note: When lifting the FAMOS Well Plate Microautosampler from the shipping container, make sure that the unit is kept upright. Lift the unit by placing your hands under the microautosampler.

If there is external damage to the shipping box, the damage should be reported to the shipping agent and LC Packings upon receipt of the goods. If internal damage is observed or if any items are missing, this should be reported to the shipping agent and to LC Packings as soon as it is observed.

Note: If there is any apparent damage to the instrument, the user should investigate the nature of the damage before plugging the unit into the mains to ensure that powering up of the instrument will not create a hazardous condition or damage internal components. If the damage appears significant, call LC Packings or its local representative before connecting the unit to the mains.

2.3 The Standard Microautosampler Configuration

A number of components of the FAMOS Well Plate Microautosampler are factory-installed as presented in TABLE 2-1. The standard tubing configuration is presented in TABLE 2-2.

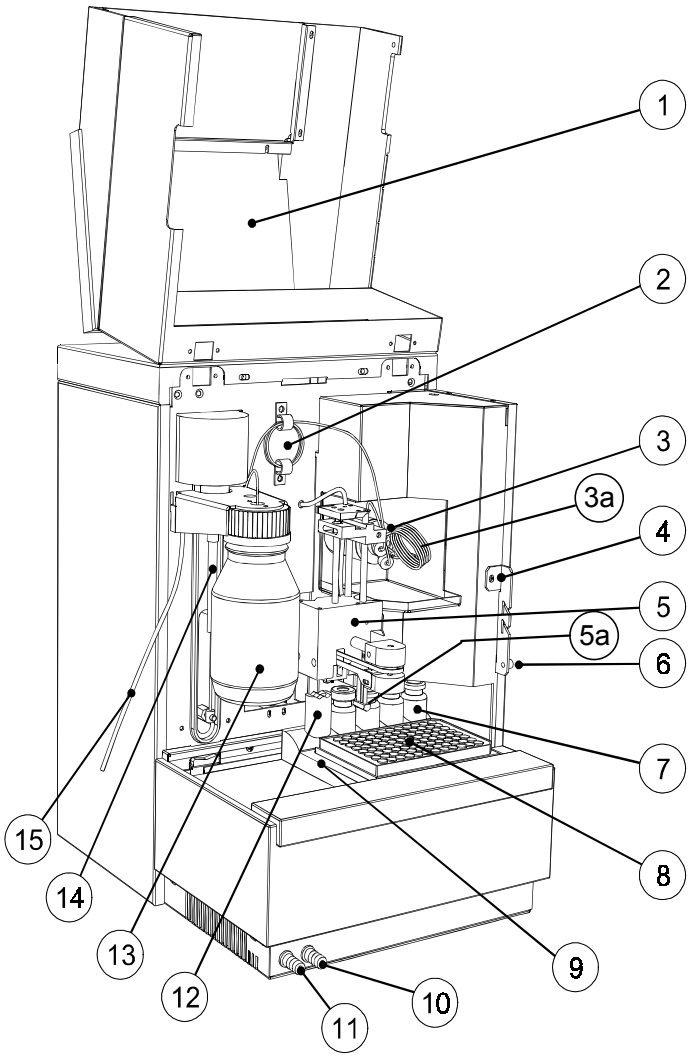
If desired, these components can be replaced to meet the specific needs of the analysis; a complete list of accessories and consumables is presented in Section 6.6. Changing the components is described in Section 6.3. Refer to FIGURE 2-1 to identify the various components.

TABLE 2-1. Factory Installed Items

Item	Description
Injection Loop	5 µL
Syringe	25 µL
Buffer Tubing	50 µL
Sample Needle	Fused silica, 2.4 µL
Wash Solvent Bottle	100 mL
Fuses (in power switch)	115 V (AC) ± 10%: two 5 AT fuses (slow, ¼" x 1¼", UL/CSA) 230 V (AC) ± 10%: two 2.5 AT fuses (slow, 5 x 20 mm, IEC127) (The fuses used are UL-listed and CSA-certified)

TABLE 2-2. Standard Tubing Configuration

Tubing	Material and Dimensions
Standard sample needle and tubing	Fused silica tubing; 300 mm x 0.280 mm O.D. x 0.100 mm I.D. (total volume 2.4 µL)
Buffer tubing from high pressure valve to syringe valve	PTFE tubing; 255 mm x 1/16" O.D. x 0.5 mm I.D. (volume 50 µL)
Tubing syringe valve to wash solvent bottle	PTFE tubing; 300 mm x 1/16" O.D. x 1.0 mm I.D.
Tubing syringe valve to waste	PTFE tubing; 400 mm x 1/8" O.D. x 1.6 mm I.D.



1 Cover (in open position)
2 Buffer tubing
3 Injection valve including the Sample Loop (3a)
4 Tubing holder
5 Needle unit with Sensor (5a)
6 Screw to fasten cover
7 Transport/reagent vials
8 Plate
9 Plate holder
10 Drain wash-position
11 Condensed water and leakage
12 Wash position
13 Wash solvent bottle
14 Syringe
15 Syringe waste tubing

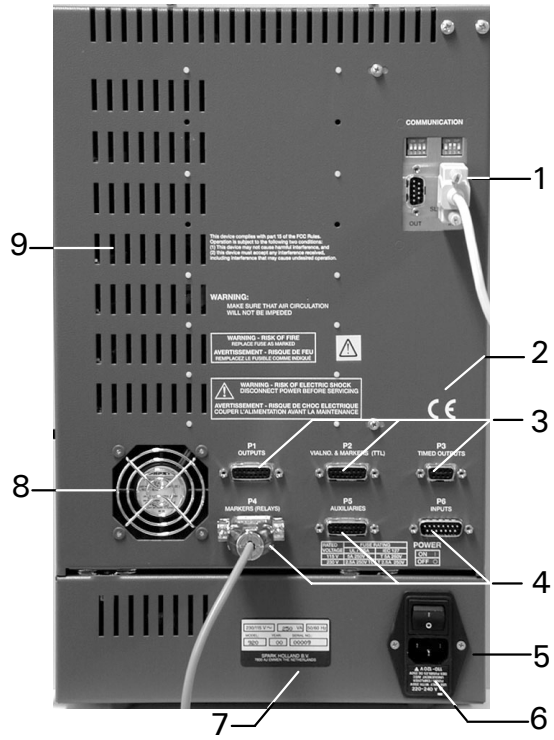
FIGURE 2-1. Front View of the FAMOS Well Plate Microautosampler

2.4 Electrical Connections

2.4.1 Inputs and Outputs

The FAMOS Well Plate Microautosampler has six standard I/O connectors (P1 - P6), five OUTPUT connectors and one INPUT connector. The Communication connector is a standard RS-232 communication interface connector. The different configurations of the I/O connectors are described in Appendix E.

All electrical connections are made on the rear panel of the autosampler (FIGURE 2-2).



1 Serial Communication Connectors (RS232)
2 CE-mark
3 I/O connectors P1 - P3
4 I/O connectors P4 - P6
5 Power Entry Module with Main Switch
6 Fuses and voltage selector
7 Type label
8 Fan (if plate cooling is installed)
9 Ventilation holes

FIGURE 2-2. Rear Panel of the FAMOS Well Plate Microautosampler

CAUTION

Caution: Avoid touching the electrical contacts on the terminal strips. Electrostatic discharges could damage internal components.

CAUTION

Caution: The manufacturer will not accept any liability for damages directly or indirectly caused by connecting the FAMOS Well Plate Microautosampler to instruments which do not meet relevant safety standards.

The electrical connections that are required depend on the nature of the instrumentation and the desired application. In this section, we describe a standard installation with other LC Packings instrumentation.

If the system is interfaced to equipment from other manufacturers, the user should refer to Appendix E for a detailed discussion of the various inputs and outputs that are provided.

2.4.2 P4 Connector – MARKERS

If the microautosampler is installed with an *UltiMate* system, the P4 connector should be connected to the START IN input of the UV Detector on the rear panel of the *UltiMate* system. To connect the P4 MARKERS outputs, use the Inject Marker Cable.

2.4.3 P5 Connector - AUXILIARIES

If the microautosampler is installed with a Switchos II™ Advanced Micro Column Switching Unit and the *UltiMate* system, the position of micro valves of the Switchos II as well as the gradient start are controlled via the P5 connector.

To connect the P5 AUXILIARIES outputs to the Switchos II and the *UltiMate* system, use the (special) interface cable (P/N 160171).

2.4.4 P1, P2, P3 and P6 Connectors

This connectors are not used during an standard installation with the *UltiMate* system (refer to Appendix E for more details when the microautosampler is used with other systems).

2.4.5 Communication Connector

The FAMOS is equipped with two 9 pin RS-232 serial interfaces. In the standard setup only the male type connector (S2) is used for digital transfer between the autosampler and the PC (item 1, FIGURE 2-2). Some communication parameters can be changed via two DIP switches above the RS-232 connectors. These switches should be set to the default settings presented in FIGURE 2-3 and should not be changed.

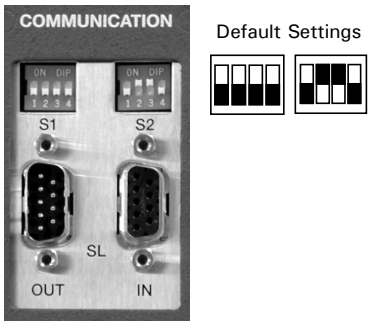


FIGURE 2-3. Default DIP Switch Settings

Note: Older versions of the FAMOS Well Plate Microautosampler are equipped with only one 25 pin connector instead of two 9 pin connectors. If the autosampler includes the DIP switches, they should be set to the default settings presented and not be changed.

2.4.6 Power Connector

The FAMOS Well Plate Microautosampler is equipped with a power supply for input voltages from 110 to 120 VAC and 220 to 240 VAC. Changing of the setting is only required if the voltage indicated by the voltage selector (FIGURE 2-4) on the back panel does not match with the mains voltage (FIGURE 2-4 indicates the setting for 220-240 V operation). The voltage indication plate can be removed with a small screwdriver.

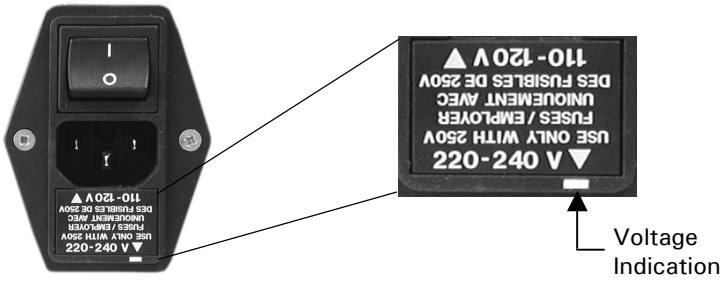


FIGURE 2-4. Power Entry Module

The power cord should be inserted in the socket directly below the Main Power switch.

- Check whether local voltage matches voltage indicated on back panel of the FAMOS Well Plate Microautosampler.
- Connect the power cord to the FAMOS Well Plate Microautosampler (item 5, FIGURE 2-2).
- Switch the FAMOS Well Plate on by using the switch at the back panel (item 5, FIGURE 2-2).

CAUTION

Caution: Make certain that the instrument is properly grounded to a true earth ground. Connecting the instrument to an ungrounded power line can cause injuries and/or damage the instrument.

2.5 Connecting the Autosampler to the HPLC System

2.5.1 Preliminary Operations

To install the FAMOS Well Plate Microautosampler to an HPLC system:

- Place the FAMOS Well Plate Microautosampler in its operating location, preferably on the left-hand side of the HPLC system. Make sure the ventilation holes are not obstructed. Allow the instrument to acclimatize for 1 hour.

- Loosen the screw at the right-hand side of the cover (item 6, FIGURE 2-1) and lift the cover so that you can perform the procedures described in this chapter.

- Install the Plate Holder (item 1, FIGURE 2-5) in the FAMOS Well Plate Microautosampler. It should be placed on the cooling plate (item 3, FIGURE 2-5) underneath the wash solvent bottle, as far to the left and to the back as possible as shown in FIGURE 2-5. The gear wheel (item 2, FIGURE 2-6) must fit against the teeth of the plate holder (indicated in white in the center of FIGURE 2-6).

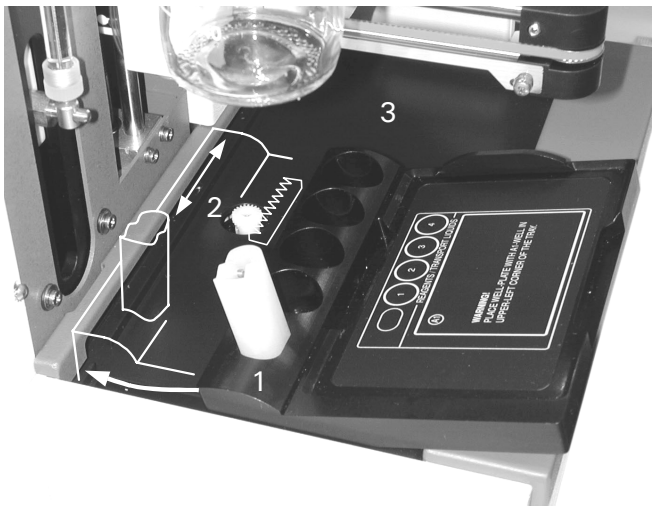


FIGURE 2-5. Installing the Plate Holder

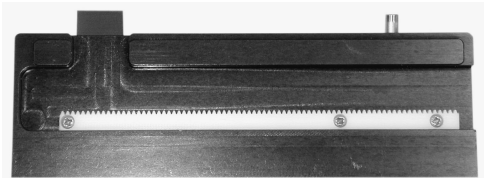


FIGURE 2-6. Bottom View of Plate Holder

- Connect the HPLC pump to port 1 of the VICI-Valco injection valve.

- Connect HPLC column to port 6 of the VICI-Valco injection valve.

Note: The instrument has been flushed with isopropanol before shipment from the factory. Make sure that the mobile phase of your HPLC system is miscible with isopropanol, or start up with an intermediate solvent as mobile phase (disconnect the HPLC column when flushing).

Note: It is very important that the contents of the sample loop are injected in back flush mode onto the column, therefore: do not exchange column and pump connections at the injection valve.

- Power up the system. The display will indicate that the self-test and initialization have been executed. After completion of this procedure, the screen shown in FIGURE 2-7 will be presented.

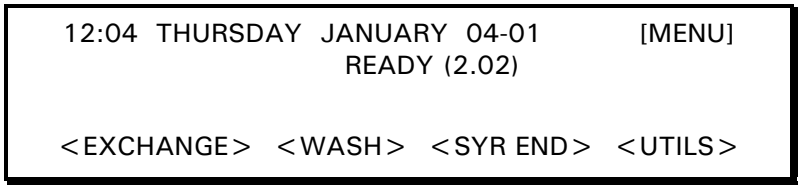


FIGURE 2-7. The Ready Menu

2.5.2 Connecting Waste Tubing

To connect the three waste tubes:

- **Syringe waste** - put the end of the syringe waste tube (item 15, FIGURE 2-1) in a bottle placed next to the FAMOS Well Plate Microautosampler.

- **Drain wash-position** - connect the hose to the drain wash connector of the FAMOS Well Plate (item 10, FIGURE 2-1); place the other end of the hose in a bottle placed on the floor. All liquid dispensed to waste at the back of the plate is removed through this drain.

- **Condensed water and leakage** - connect the hose to the drain port of the FAMOS Well Plate (item 11, FIGURE 2-1). Place the other end of the hose in a waste container on the floor. All leakage of solvents and condensed water are drained through this hose (if Peltier cooling option is installed).

Note: Make sure that the flow path of the hoses is not obstructed in any way.

2.5.3 Filling the Wash Solvent Bottle

The FAMOS Well Plate Microautosampler is shipped with a 100 mL wash solvent bottle.

To install the wash solvent bottle:

a) Fill the wash solvent bottle with the appropriate wash solvent. Use of mobile phase in case of isocratic separations and mobile phase A – without any salt or modifiers added to it – in case of gradients is recommended. Before using the wash solvent, degas it with Helium or an ultrasonic bath, or degas it on a continuous basis.

b) Screw the bottle to the cap in the holder.

c) Place the holder in the microautosampler as indicated in FIGURE 2-8.

d) Put the wash solvent tube in the wash solvent.

e) Flush the syringe (Section 2.5.4).

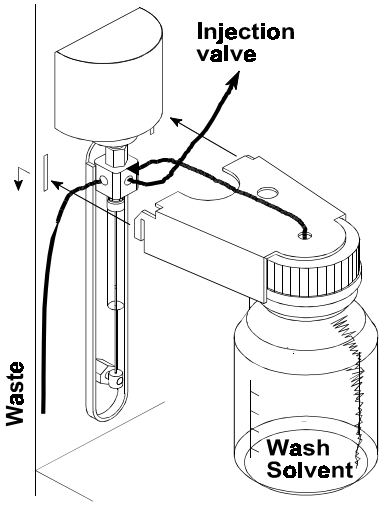


FIGURE 2-8. Wash Solvent Bottle

If you use an application that requires more than 100 mL of wash solvent for a complete run, use a 250 mL wash solvent bottle (P/N 162033) or install a longer tube (with flanged end for valve fitting) and place a larger bottle next to the FAMOS Well Plate Microautosampler. To fill the wash solvent tube, you may have to repeat the flushing procedure a few times.

2.5.4 Flushing the Syringe

The FAMOS Well Plate Microautosampler is supplied with a 25 µL syringe. It is also possible to use the microautosampler with a 100 µL, 500 µL, 250 µl or 1000 µL syringe. Refer to Section 6.3.1 for instructions how to replace the syringe.

To remove the air and flush the syringe:

a) Check the general settings in the System Menu (Section 3.8) and make sure that the appropriate syringe and the corresponding buffer tubing is installed.

b) Select soft function key <WASH> in the Ready Menu to perform a standard wash routine. All tubing connected to the syringe valve is filled and rinsed.

c) If any air remains in the syringe, perform an additional wash cycle and gently tap the syringe as wash solvent is dispensed to waste.

Note: If there is still air in the syringe gently tap the syringe as wash solvent is dispensed to waste during the wash cycle.

2.5.5 Adjusting the Needle Height

The sample needle height can be programmed within the general system settings (used for injection methods only), the mixed methods and within user defined programs (Chapter 3). This parameter is defined as the distance from the top of the plate holder to the bottom of the sample needle when the sample needle is moved all the way down.

This parameter can be adjusted to insert the sample needle as far as possible into the sample vial to aspirate the whole sample out of the vial (FIGURE 2-9). The default setting is 2 mm.

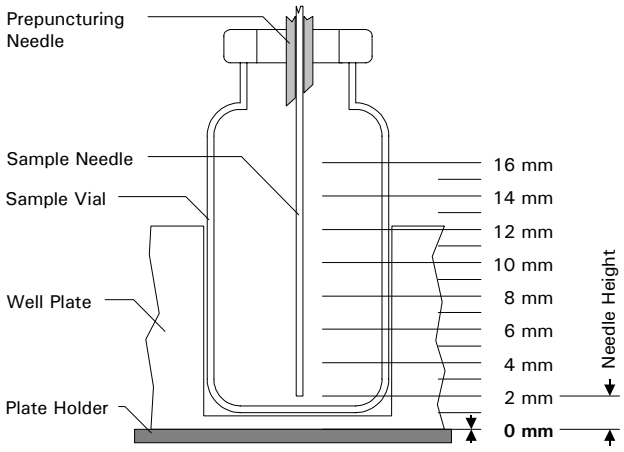


FIGURE 2-9. Positioning the Sample Needle

CAUTION

Caution: A sample needle height '0' corresponds to the top of the plate holder! Operating the FAMOS Well Plate Microautosampler with this value set to '0' and with a well plate installed at the same time may damage the sample needle. This is especially true if the conventional stainless steel needle is used.

2.6 Routine Operation of the System

2.6.1 Sample and Mobile Phase Considerations

The FAMOS Well Plate Microautosampler is used in an HPLC system and the "standard" operating precautions for HPLC should be employed:

- Ensure that samples and mobile phases do not contain particulate matter. All samples and mobile phases should be filtered through a 0.22 μm membrane filter. If organic solvents are used, make sure that extractable materials are not present in the filter.
- The sample should be soluble in the mobile phase. If a gradient is used, make certain that the sample is soluble in the mobile phase at all mobile phase compositions to be used in the separation.
- After you have finished using the system, flush it with a water/methanol or water/acetonitrile mobile phase before shutting it down.
- Solvent should be degassed by sparging with He.

2.6.2 Plates and Sample Handling

The FAMOS Well Plate Microautosampler accommodates the following types or plates:

- 96-low wells
- 96-high wells
- 384-low wells
- 48-vials

A sensor (item 5a, FIGURE 2-1) monitors plate detection, plate height detection and vial detection.

Because the FAMOS Well Plate Microautosampler uses headspace pressure during sample injections, it is very important that samples are properly handled. Note the following:

- Standard wells can best be filled by means of a narrow-end pipette to allow air to escape when filling the well.
- If wells are filled to the rim, the sample might be forced into the prepuncturing needle, causing cross-contamination of samples and contamination of the sample needle.
- If vials are used (with a plate for vials), make sure the seals are airtight to prevent air bubbles in the sample and prevent evaporation of volatile samples; check seals after crimping; if the cap can be turned easily, the seal is not airtight and the handcrimper should be adjusted.

Note: If wells that are not airtight are used, switch off the headspace pressure in the System Menu (General Menu).

Note: Check whether the needle height is sufficient for the new type of plate that is installed and adjust if necessary (System Menu, General Menu).

To replace a plate in the FAMOS Well Plate Microautosampler:

a) Select the <PLATES> soft key in the Ready Menu, then select <EXCHANGE>. The plate moves to the left.

b) Take out the plate and replace it by another one.

c) Select the <PLATE HOME> soft key. The plate moves to operating position again.

d) If you have replaced the plate by a plate of the same type, you are now ready. If you have installed a new type of plate, execute the following steps:

- Press **System**.
- Select the <PLATES> soft key.
- Press **E**.
- Select the soft function key for the type of plate concerned.
- Press **E** and determine whether to process the plate in <ROWS> or in <COLUMNS>.
- Press **Escape** twice to return to the Ready Menu.

A message appears to indicate that all programmed series will be reset. The user will have to redefine series because the settings in the System Menu have been altered.

2.6.3 Placing Reagent Vials/Transport Vials in Position

To replace reagent vials/transport vials:

a) Select the <PLATES> soft key and then select <EXCHANGE> in the Ready Menu. The plate holder moves to the left.

b) Take out the reagent vials/transport vials (item 7, FIGURE 2-1) and replace them by other reagent vials/transport vials.

c) Select the <PLATE HOME> soft key. The plate moves to operating position again.

e) Press **Escape** twice to return to the Ready Menu.

Note: Reagent and transport vials can be placed in any of the four positions. Transport vials must be placed in a continuous row.

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CHAPTER 3

The User Interface

3.1 Overview

This chapter describes the general mode of operation of the FAMOS™ Well Plate Microautosampler and explains how an operating program is established. It includes a discussion on:

- Powering Up the Microautosampler (Section 3.2)
- A description of the front panel and the role of the keys on the front panel (Section 3.3)
- Menus of the FAMOS Well Plate Microautosampler (Section 3.4)
- General approach to entering and executing a program (Section 3.5)
- Types of Methods (Section 3.6)
- Executing a Series (Section 3.7)
- Menus and Operating Commands (Section 3.8)
- Series Menus (Section 0)

Typical programs are presented in Chapter 4 and Appendix C.

3.2 Powering up the FAMOS Well Plate Microautosampler

a) When the FAMOS Well Plate Microautosampler is powered up via the main power switch on the rear panel, it will go through an initialization/self-test protocol. During this period, a number of messages are displayed indicating that various components are functioning properly.

b) After completion of this procedure, the **Ready Menu** appears on the display:

```
14:46  THURSDAY  JANUARY 04-01          [MENU]
                 READY (2.02)

   <PLATES>  <WASH>  <SYR END>  <UTILS>
```

FIGURE 3-1. The Ready Menu

Note: In this manual, the various display messages and menus shown correspond to a system with firmware version V2.02. If a different version of the firmware is used, there may be small differences in the screens and/or actions that occur when a given command is performed.

3.3 The (Upper) Front Panel

Communication between the user and the system is provided by the keypad on the front panel of the FAMOS Well Plate Microautosampler (FIGURE 3-2) or via the *UltiChrom*™ Software. This section describes the display and the use of the keypad when the microautosampler is used on a local basis (i.e. not controlled by *UltiChrom* Software).

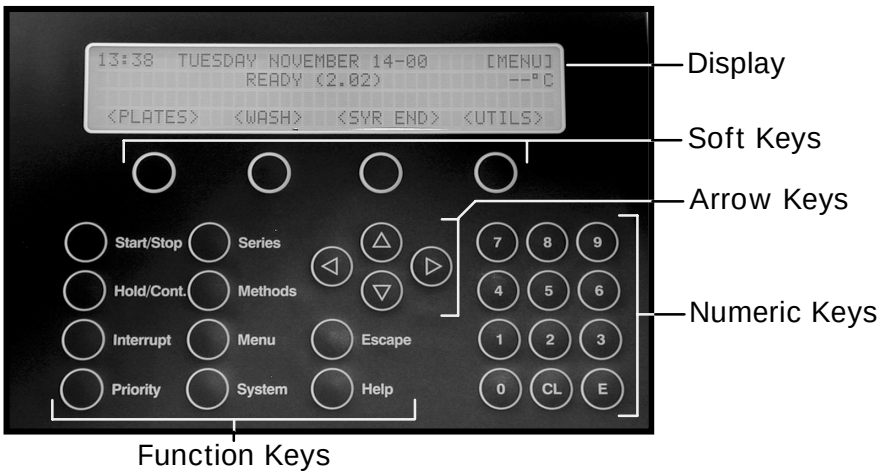


FIGURE 3-2. Keypad and Display

The front panel includes:

- **Display** - indicates a variety of system parameters.
- **Soft Keys** - the label assigned to these keys depends on the menu that is active. The current function of each key is shown in the bottom line of the display.
- **Arrow keys** - used to move to a different field in the display, to move to a different field in a menu, or to change the value of a field.
- **Numeric Keys (0** to **9)** - used to enter values in the various programming fields.
- **CL**(Clear) - used to clear a value in a field or replace it by NONE or AUTO.
- **E** (Enter) - used to accept the present value or setting for an entry. The entered value is checked for validity and then saved.

Function keys:

a) Run control keys:

- **Start/Stop** - used to start or stop automatic processing (and data acquisition when controlled via the output connectors) or to reset the system after an error has occurred.
- **Hold/Cont**.- used to hold or continue the analysis time. The analysis time is extended by the period that Hold is active.

- **Interrupt** - not used

- **Priority** - used to stop a run so that a priority sample is run before analyzing the rest of the programmed sample series. Before the run is interrupted, processing of the present sample will be finished. As soon as the priority sample has been analyzed, the analytical run is resumed. A priority sample is a series of one well with an injection method, a wash method and a time base method defined in a template (this run is possible only if the correct settings are entered in the System Menu).

b) Programming keys:

- **Series** - used to enter the Series Menu in which series can be defined for an analytical run.

- **Methods** - used to enter the Methods Menu in which methods can be programmed for use in an analytical run.

- **Menu** - this key can only be used if [MENU] or [MN] is shown in the top right hand corner of the display. If this key is pressed, additional fields of the menu are displayed.

- **System** - used to enter the System Menu in which system settings can be entered.

c) General keys:

- **Escape**- allows the user to leave the programming mode or go to a previous level in the menu. A value that is entered is checked for validity and then saved.

- **Help** - used to display help information (which is available only for a limited number of functions).

3.4 Menus of the FAMOS Well Plate Microautosampler

The software of the FAMOS Well Plate Microautosampler is menu-driven. In this section, we present an overview of the menu structure, as well as a detailed description and a menu reference (name) for each command (Section 3.8). In addition, a detailed programming flow chart is presented in Appendix B).

To access a menu, click on the soft key that corresponds to the menu. A few menus have a sub menu associated with it; in these cases, the desired menu is chosen in the standard way after the top level menu is accessed. When you are in a menu, various items are accessed, and you should indicate the appropriate choice(s) to generate the desired method or series.

The microautosampler menus are:

- **Ready Menu** - this menu appears when the FAMOS Well Plate Microautosampler has been powered up and passed the initialization test. It includes basic system commands (e.g. washing and plate handling), a number of utilities (e.g. copying a method) and commands to establish communication with other systems.

- **System Menu** - this menu is used to select settings that are changed infrequently (e.g. the loop volume, the type of plate, the system clock, if the alarm should be set, etc.). In some cases, these settings may have been made at the factory to suit particular wishes of the user; in this instance, it will not be necessary to make changes. It is recommended that you enable only those facilities that you will actually need; this will ensure that menus are as concise as possible.

- **Methods Menu** - this menu is used to program a method to be used in an analytical run and to assign a number to it. Typical commands include the analysis time, the wash volume and wash parameters.

- **Series Menu** - this menu is used to define a series, and to assign a number and a method to it for the analytical run.

If [MENU] or [MN] is displayed in the top right hand corner of the screen, you can press the **Menu** key on the keypad to display more possibilities offered by the menu. An explanation of all keys on the keypad is presented in Section 3.3.

3.5 General Approach to Entering and Executing a Program

After you have determined the type of analytical run that you want to perform, the best way to generate the order of operation for the FAMOS Well Plate Microautosampler is:

a) Enter the desired settings in the **System Menu**. It is probable that the settings in the System menu have been correctly entered (factory-installed) as these settings are global in nature.

b) Program a method for the analyses you wish to perform via the **Methods Menu**.

c) Define a series and link a programmed method to a range of wells in the **Series Menu**.

d) Execute the series.

While you could use a different order than that indicated above, it should be noted that some of the settings in the System Menu define the options in other menus. If an invalid selection is made on the System Menu, the desired option for other menus may not be presented. As an example of this point, the USER PROGRAM menu (which is defined in Section 3.8.14) will be presented only if the USER PROGRAM option has been selected in the System Menu (Section 3.8.2).

3.6 Types of Methods

The FAMOS Well Plate Microautosampler provides the following types of methods for different aspects of the operation of the microautosampler:

- **injection** method - contains information on the injection routine, flush volume and analysis time.
- **wash** method - describes a wash volume and when a wash must be executed.
- **mix** method - a pre-injection method in which additional sample handling can be performed (e.g. pre-column derivatization).
- **timebase** method - a post-injection method with which outputs to other devices (e.g. integrator or pump) and switching of the ISS valve are controlled.
- **user program** - offers the possibility to program sequences of all actions that can be executed by the FAMOS Well Plate Microautosampler in separate steps.

Each programmed method is assigned a number. The FAMOS Well Plate Microautosampler allows you to store a combination of defined methods in a **template**, which is also identified by a number.

Methods must be linked to a series before they can be used. The following options are available with the FAMOS Well Plate Microautosampler:

- You can assign an individual **method** to a series: methods (mix, injection, wash, timebase) can be linked to wells in a series.
- You can assign a **template** to a series: a combination of various programmed methods (mix, injection, wash, timebase) can be defined in a template. The template is linked to a range of wells in a series. In this way, all steps in an analytical run are defined and stored.
- You can assign a **user program** to a series: This can be used to combine all possible steps in the analytical process in one program. The user determines the order of the separate actions the FAMOS Well Plate Microautosampler has to perform.

3.7 Executing a Series

3.7.1 Manually Executing a Series

A series can be run from the Ready Menu by pressing the **Start/Stop** key. Execution of a series is possible if you have programmed a method and defined a series for the samples you wish to analyze.

Note: Series are not stored in battery backup memory and exist only for as long as the FAMOS Well Plate Microautosampler is powered up.

To execute a series:

a) Start programming the series by pressing **Start/Stop**.

b) Enter the number of the first series to be performed and press **E** (to confirm the input and go to the next screen), then indicate the number of the last series to be performed and press **E** again.

c) After the range of series is defined, the autosampler will ask for the start conditions. It is possible to start the series either from the keypad or by remote control inputs (NEXT INJECTION and NEXT VIAL inputs, connector P6).

d) Select <START> to manually start the analytical run. The FAMOS Well Plate Microautosampler will begin execution of the series that you have defined.

During the run, the display shows information about the current operation.

Note: Series are always executed in numerical order. Empty series will be skipped.

You can stop a run by pressing the **Start/Stop** key. The FAMOS Well Plate Microautosampler will execute a shut down sequence by removing all sample from the buffer tubing and performing a wash routine.

If you want to stop the microautosampler immediately (panic stop), without performing a buffer clean up; press the **Start/Stop** key twice. Remember to perform a wash routine to clean up the tubing before you start the FAMOS Well Plate Microautosampler again.

CAUTION **Caution: A panic stop does not remove the sample from the buffer tubing. Before starting again, you should perform a wash sequence.**

After the FAMOS Well Plate Microautosampler has completed the run, the Ready Menu will appear again.

A number of examples of the use of a series are presented in Chapter 4 and Appendix C.

It is possible to program a series and/or a method during a run by pressing **Series** or **Methods.** The menus that are presented in this mode are identical to those offered when the FAMOS Well Plate Microautosampler is idle.

If a series or method is changed, the new values become active the next time the FAMOS Well Plate Microautosampler initiates a series. The series currently running are not affected by the changes.

3.7.2 Executing a Series via Remote Control

After the range of series has been programmed, the FAMOS Well Plate Microautosampler will ask for the Start conditions. If you press the <REMOTE> soft key, the Remote mode of operation will be selected. In this mode, the autosampler will act as a slave of another device (e.g. an HPLC pump) and can be controlled with the NEXT INJECTION and NEXT VIAL inputs of connector P6 (see Appendix E).

- **NEXT INJECTION INPUT** - this input will start the next injection of the series. If the series was completed, it starts the next injection from the next series, until all series is completed.

- **NEXT WELL INPUT** - this input will start the first injection from the next vial in the series. If the series was completed, it starts the first injection from the first vial of the next series, until all series are executed.

Note: The difference between the NEXT INJECTION and the NEXT WELL INPUT is that the NEXT WELL INPUT directly starts the first injection from the next vial, even if not all programmed injections from the previous vial were executed.

To indicate that the remote control mode is activated, the letter “r” is displayed in the lower left corner of the display.

To execute a series via remote control, execute the following steps:

a) Press **Start/Stop**.

b) Enter the number of the first and the last series to be performed (each entry should be confirmed by the **E** button).

c) Select <REMOTE> to enter the remote control mode. The FAMOS Well Plate Microautosampler will now operate as slave of another device and can be controlled with NEXT INJECTION INPUT or NEXT WELL INPUT.

d) Press **Escape** to return to the Ready Menu.

At the end of the series, the message “Series completed via remote control” is displayed.

3.8 Menus and Operating Commands

This section describes all the features and possibilities of the soft keys included in the FAMOS Well Plate Microautosampler firmware, in the order in which they appear on the screen. An overview of the programming scheme is presented in Appendix B.

The different levels of the soft function keys (commands) on the different menus are indicated as follows:

< PLATES >	(**< bold with brackets >**) represents the top level.
< EXCHANGE >	(< normal type with brackets >) represents second level.
NEEDLE HEIGHT	(normal type) represents the different parameter names.

3.8.1 Ready Menu

The Ready Menu contains the soft function keys presented in FIGURE 3-3.

Overview

< PLATES >	**< WASH >**	**< SYR END >**	**< UTILS >**
Second page of the [Menu]			
< SERIAL >	**< SSV > ***	**< COOL > ***	**< SERVICE >**

*) Only if option is installed

FIGURE 3-3. The Ready Menu

All available soft function keys and options on the Ready Menu lead to the following menu:

< PLATES >	This key is used to access commands that are used to exchange well plates.
<EXCHANGE>	Press < EXCHANGE > to move the plate to the left; in this position the plate can be replaced without damage to the equipment.
<PLATE HOME>	Press the soft function key < PLATE HOME > to move the plate to operating position again.

< WASH >	This key is used to start a standard wash procedure. All tubing connected to the syringe valve will be filled and rinsed with wash solvent.

< SYR END >	This key is used to move the syringe to the end position (e.g. if you wish to replace the syringe needle or to simplify filling of wash solvent tubing). A syringe volume of wash solvent is aspirated from the wash solvent bottle and the wash solvent tube is filled. When the key is selected, it is redefined to < SYR HOME >, which is used to dispense the syringe contents to syringe waste and to move the syringe to the standard operating position again.

< UTILS >	This key is used to enter the Utilities Menu.
	Note: If a method protection code is enabled in the System Menu, the code must be entered to access the Utilities Menu.
< COPY >	This key is used to copy a method, select the method type (mix, injection, timebase, wash), the number of the method to be copied and enter the number for the destination method. An existing method stored under that number will be overwritten.
< ERASE >	This key is used to erase a method (Template, Methods, User Program). If Template and User Program are disabled in the System Menu, the soft function keys for erasing a standard Method (mix, injection, wash, timebase) appear. It is not possible to erase the user program if the protection code for the user program is enabled in the System Menu.
< LOG >	The FAMOS Well Plate Microautosampler keeps a log of system-relevant events (< EVENTS > ; records error messages that have been generated) and keeps count of actions of valves and syringe movements (< COUNT >). The message "**Lifetime of syringe (valve) may be exceeded. Check for possible leakage!**" appears after every 50,000 syringe actions and after every 200,000 syringe valve actions.
	Note: If you do not replace the Syringe when the message is presented, and tell the system "not to display this message again", the message will not be displayed again until an additional 50,000 more syringe actions have been counted.
	Note: If the Syringe valve is replaced, the counter should be reset to zero by the service engineer.
< DEFAULT ALL >	This key is used to change all software settings to default. All series, methods, templates and the user program (unless protected by protection code) will be erased.
	Note: If < DEFAULT ALL > is selected, check whether the hardware configuration is still compatible with the settings entered in the System Menu.

< SERIAL >	This key is used to set the FAMOS Well Plate Microautosampler to serial mode.
	Note: If a method protection code was defined in the system settings, the code must be entered to get access to serial mode.
< PANIC >	This key is used to initiate a stop sequence. A panic stop does not remove the sample from the buffer tubing. Before starting again, you should perform a wash sequence in which all tubing is rinsed and the valve and I/O ports are reset. At the end of the sequence, serial mode operation is resumed.
< EXIT >	Press to end serial mode and return to the Ready Menu.

< SSV > (option)	This soft key is used to start a procedure in which all lines of the solvent selection valve can be primed (if this option is included in the system).

< COOL > (option)	This soft key is used to enter the programming mode for Peltier plate cooling (if this option is included in the system). If the cool option is switched < ON >, the following soft function keys can be selected:
< MANUAL >	The temperature control will remain OFF until it is switched on again by the user (in this menu).
< AUTOMATIC >	The temperature control will be switched OFF after all programmed series have been executed.
< DATE-TIME >	The temperature control will be switched OFF at a date and time that can be user selected.
	Note: The programmable temperature range is 4°C to 40°C. The maximum cooling capacity is approximately 20°C below ambient. Make certain that the condensed water and leakage tubes are connect to a waste container on the floor to drain condensed water.
	Note: The temperature of the sample inside the sample vial may be slightly different than the temperature set, due to a variety of effects (e.g. the heat transfer characteristics of the vial walls). If it is necessary to precisely set the temperature of the sample, we recommend that you determine the temperature inside the vial at various temperature setpoints and set the cooling option so that the desired internal temperature is attained.

< SERVICE >	This key is used to access a variety of service related commands and it is protected by a code. Use of these commands is restricted to authorized service personnel.

3.8.2 The System Menu

When you press the **System** key, the display asks whether the autosampler should operate in the < MICRO > or in the < CONVENTIONAL > mode.

< MICRO >	< CONVENTIONAL >

The ranges and the default settings of some system parameters are dependent on this selection of the mode of operation.

Note: If you change from one operating mode to the other, all parameters will be set to their default values for that mode.

In both operating modes the System Menu offers the soft function keys presented in FIGURE 3-4 and which are discussed in detail in Sections 3.8.3 – 3.8.8 (< CLOCK > and < COMM > are available after pressing the **Menu** key).

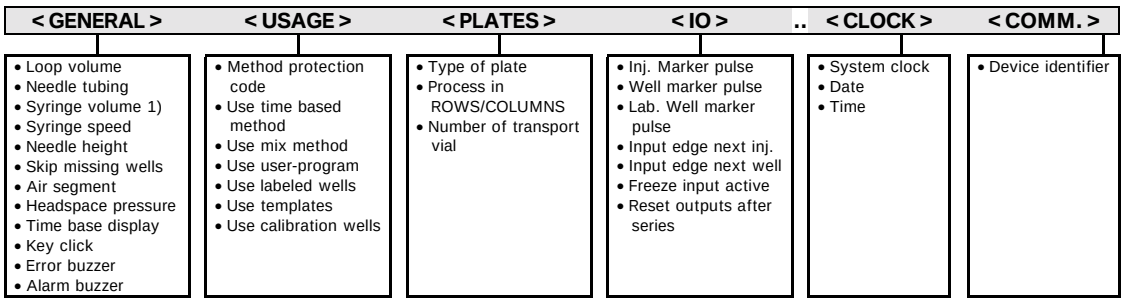


FIGURE 3-4 The System Menu

3.8.3 The System Menu Item < GENERAL >

Note: The microautosampler can be fitted with different syringes, tubing lengths, etc. to meet the specific needs of the analysis. The General Menu is used to enter the system configuration; it is critical that they are entered so that various operations are performed properly (e.g. the minimum flush volume is dependent on the needle volume). Once these settings are established, they are not changed frequently.

< GENERAL >	This soft function key is used to access the following parameters:
LOOP VOLUME NEEDLE TUBING SYRINGE VOLUME	These values are entered by the user so that the system can determine the appropriate values for certain operations. As an example, the minimum flush volume is dependent on the specific needle and needle tubing. The default flush volume equals two times the volume of needle and tubing.
SYRINGE SPEED SCALE FACTOR	The maximum aspiration speed of the syringe used in injection methods is dependent on the viscosity of the samples. As an alternative, the syringe speed can be reduced by entering a scale factor. The actual syringe speed will be the scale factor multiplied by the syringe speed. The speed of the syringe during the wash or the rinsing procedure of the buffer are not affected by this setting.
...	

NEEDLE HEIGHT	This parameter refers to the distance between the needle point and the plate holder (default: 2 mm). The value is only used in injection methods (for mix methods this value is programmable in the method itself).
SKIP MISSING VIALS	Appears only if a plate with 48 vials is selected in System Menu (Plates Menu). YES means that empty spaces are skipped during the run. NO means that the Microautosampler will stop if an empty space is observed during the run and an error code will be generated.
AIR SEGMENT	This command is used to indicate whether an air segment will be used for analytical runs (for explanation of air segmentation refer to Appendix A).
HEADSPACE PRESSURE	This command is used to indicate if the headspace pressure should be on or off. The FAMOS Well Plate Microautosampler uses headspace pressure to facilitate transport of sample into the loop. The compressor will always be used during a wash procedure. ☞ **Note: Accuracy and reproducibility may decrease if headspace pressure is switched off. However, headspace pressure will only be useful if sample wells are airtight**
TIME DISPLAY	This command is used to offer a choice between two modes of representing the time.
KEY CLICK ERROR BEEP ALARM BUZZER	These commands are used to allow the user to present a audible sound when a key is pressed, when an error is observed or if an alarm is observed.

TABLE 3-1 presents an overview of the default general settings and their ranges.

TABLE 3-1. Overview of General settings

< GENERAL > Parameter	**Default Setting**		**Range**	
	Micro	Conventional	Micro	Conventional
Loop volume	5 µL	100 µL	1 - 20 µL	5 - 1000 µL a)
Needle tubing	2.4 µL	15 µL	1 – 99.9 µL	1 - 200 µL
Syringe volume	25 µL	250 µL	fixed to 25 µL	100, 250, 500 or 1000 µL
Syringe speed	low	normal	low, normal or high	
Scale factor	0.1	0.1	0.1 - 1.0	
Needle height	2 mm		0 - 40 mm	
Skip missing vials	yes		yes or no	
Air segment	yes		yes or no	
Headspace pressure	no		yes or no	
Time base display	HH:MM:SS		H:MM:SS or H:MM:mm	
Key click	on		on or off	
Error beep	on		on or off	
Alarm buzzer	on		on or off	

Note: a) 250/500 µL syringe: use 500 µL buffer tubing
500/1000 µL syringe: use 2000 µL buffer tubing

3.8.4 The System Menu Item < USAGE >

< USAGE >	This soft function key is used to access the following settings:
PROTECTION CODE	This entry is used to enter a code for protection of methods. A six digit code (000000-999999) can be selected and is erased by pressing **CL**. If a code has been defined, it is not possible to enter the System Menu and the programming menus without entering the protection code. Default: none.
TIMEBASE METHODS	This entry is used to enable/disable the ability to program timebase methods. The microautosampler controls other connected equipment during analysis time. Timebase methods are programmed via the Methods Menu. Default: disabled.
MIX METHODS	This entry is used to enable/disable the ability to program mix methods for the microautosampler. Program mix methods in accessed via the Methods Menu. ☞ **Note: The microautosampler cannot analyze priority samples during a run if the mix method is enabled. Default: disabled.**
USER PROGRAM	This entry is used to enable/disable the possibility to generate a user program. If this function is enabled, it is possible to enter a user program protection code (6 digits). A user program is generated via the Methods Menu. Default: disabled ☞ **Note: The microautosampler cannot analyze priority samples during a run if the user program is enabled.**
LABELED WELLS	This entry is used to enable/disable the possibility to program labeled wells. The location of labeled wells is programmed via the Series Menu. Default: disabled
TEMPLATES	This entry is used to enable/disable the possibility to program templates. Templates are programmed via the Methods Menu. Default: disabled.
CALIBRATION WELLS	This entry is used to enable/disable the possibility of programming calibration wells. The location of the calibration wells is entered in the Series Menu. Default: disabled. ☞ **Note: We recommend that you disable as many functions in the Usage Menu as possible to make sure that other menus do not contain possibilities that are irrelevant for the type of analyses you are to perform.**

3.8.5 The System Menu Item < PLATES >

< PLATES >	This soft function key is used to access the following settings:
TYPE OF PLATE	This entry is used to define the type of plates that will be used. Four types can be selected: 96-low (default), 96-high, 384-low or 48-vials. After a plate type has been selected, press ENTER:
WELL PROCESSING METHOD	Indicates if samples should be processed in rows (left to right) or in columns (top to bottom).
FIRST TRANSPORT VIAL	Enter a number 1 – 4, or press **CL**.
LAST TRANSPORT VIAL:	Enter a number 1 – 4.

Note: Vials can be placed in any of the four positions. Transport vials must be placed in a continuous row.

3.8.6 The System Menu Item < IO >

< IO >	This soft function key is used to access the IO configuration mode and define the following (the default and range are indicated in TABLE 3-2):
INJECT-MARKER PULSE LENGTH	This entry is used to define the length of the inject-marker pulse.
WELL-MARKER PULSE LENGTH:	This entry is used to define the length of the well-marker pulse.
LABELED WELL MARKER PULSE LENGTH	This entry is used to define the length of the well-marker pulse of the labeled well.
INPUT EDGE NEXT INJECTION	This entry is used to define the edge sensitive inputs for the next injection.
FREEZE INPUT ACTIVE	This entry is used to define whether the freeze input is active when high, or freeze input is active when low.
RESET OUTPUTS AFTER LAST SERIES	This entry is used to indicate whether the outputs should be reset to default after the last series.

Note: Refer to Appendix E for more specific information on IO connections.

TABLE 3-2. Overview of IO settings

IO Parameter	Default	Range
Inject-marker pulse length	1.0 s	0.1 - 2.0
Well-marker pulse length	1.0 s	0.1 - 2.0
Labeled well marker pulse length	1.0 s	0.1 - 2.0
Input edge next injection	falling	falling or rising
Input edge next well	falling	falling or rising
Freeze input active	low	low or high
Reset outputs after last series	no	yes or no

3.8.7 The System Menu Item < CLOCK >

< CLOCK >

This entry is used to switch the system clock on or off. If the ON selection is made, you can set date (yy:mm:dd) and time (hh:mm). This date and time will be displayed in the Ready Menu.

3.8.8 The System Menu Item < COMM >

< COMM. >

This entry to define a device identifier for communication with other equipment (e.g. a PC). An identifier between 20 and 29 can be selected for the FAMOS Well Plate Microautosampler, default setting is 21.

Note: When controlling the FAMOS Well Plate Microautosampler by *UltiChrom*, make certain that the same setting is used in the hardware description.

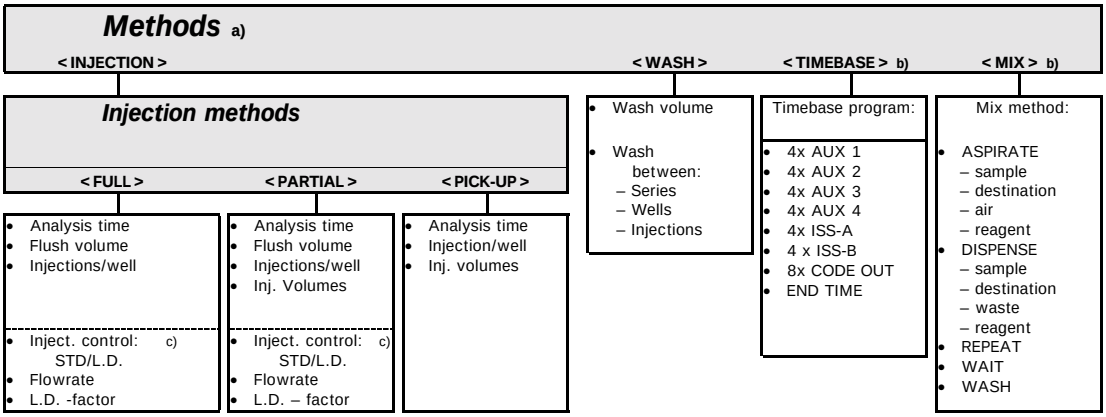
3.8.9 Methods Menu

When entering the Methods Menu (by pressing the **Method** key), the user can program various types of methods. It is possible to define up to:

- 24 Separate Injection methods
- 5 Wash methods
- 5 Timebase methods
- 9 Mix methods
- 1 User program

It is possible to program a combination of methods and save them in a template. The available parameters and options in a method or template may vary according the settings made in the System Menu.

An overview of the Methods Menu is presented in FIGURE 3-5.



Notes: a) < Template > and < User Program > disabled in System Menu
b) When enabled in System Menu only
c) In micro mode only

FIGURE 3-5. The Methods Menu

Note: The options provided by the Methods Menu depend on the settings made on the System Menu.

3.8.10 The Method Menu Item < INJECTION >

< INJECTION >

This soft function key is used to access commands to program a method that defines injection methods for full loop, partial Loop and µL pick-up operation. Up to 24 methods can be programmed.

< FULL LOOP >

< PARTIAL LOOPFILL >

< µL PICK-UP >

Select the desired injection method and then enter a number to define the injection method to be programmed. If the selected method is locked because of changes in the settings (System Menu) after programming the method, the word LOCK is displayed. The method can be unlocked by programming valid values in the method itself or by restoring the values in the System Menu.

ANALYSIS TIME

This entry defines the time between switching the injection valve to inject and the start of processing the next sample.

FLUSH VOLUME

This entry defines the amount of sample taken from a vial before the loop is filled with sample. Default value: 5.0 µL (combined with an air segment).

Note: Flush volumes of less than twice the volume of the needle and tubing will result in decreased performance.

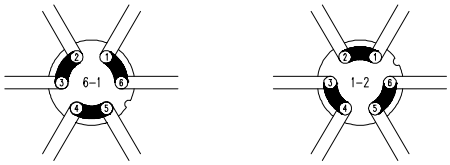
...

NUMBER OF INJECTIONS PER VIAL	This entry defines the number of injections (up to 9) per vial.
INJECTION VOLUME	Indicates the injection volume for each injection from each vial. The maximum programmable injection volume is: **partial loopfill**: 50% of the programmed loop volume **µL pick-up**: injection volume = (loop volume – 3 x needle volume)/2 **full loop**: This parameter is not programmable, and is equal to the loop volume. It requires more sample to fill the loop than other techniques.: 3 x loop volume for loop volumes < 100 µL; 2 x loop volume for loop volumes ≥ 100 µL - 499 µL; 1.5 x loop volume for loop volumes ≥ 500 µL.
INJECTION CONTROL	**Note: Injection controls options are available in Micro mode and only in conjunction with partial loopfill or full loop injection only.** Determines whether the injection is performed in the is standard (< STD >) or in the low dispersion mode (< L.D. >).
FLOW RATE	Enter the flow rate.
L.D. FACTOR	This entry is used to indicate the low dispersion factor, range 0.7 - 2.0 **Note: These two parameters determine the switching time of the injection valve, refer to Appendix A for more details.**

3.8.11 The Method Menu Item < WASH >

< WASH >	This soft function key is used to access a series of commands to program wash methods. You can program a wash between injections, samples or series. When you select a wash method, a screen will be presented that will allow you to indicate the volume of wash solvent can be defined. The minimum programmable volume is 300 µL.

3.8.12 The Method Menu Item < TIMEBASE >

< TIMEBASE >	This soft function key is used to enable control of the optional ISS valve and other devices via auxiliary (P5) or binary TTL outputs (P3).
(if enabled in System Menu, Usage Menu)	Up to 5 timebase methods can be programmed. The menu offers the following soft function keys:
< AUX >	This command scrolls through all program lines by pressing **E** or select AUX to move to the next auxiliary. There are four AUX entries (AUX1-AUX4).
< VALVES >	This command controls the ISS valve and the solvent selection valve. The ISS valve can only be programmed if the optional ISS valve is installed. The entries 6-1 and 2-1 refer to the interconnected ports of the valves. Press **E** to scroll through programming lines. Enter the time and the SSV port number (value between 1 and 6). 
< CODE >	Enter a time and a hexadecimal value between1 and 15. Press **E** to scroll through the programming lines.
< END TIME >	This command is used to enter the end time for timed events program; press **E** to scroll through the programming lines. If no value is filled in or if **CL** is pressed, the FAMOS Well Plate Microautosampler will automatically generate an end time. The end time is equal to the analysis time programmed in the injection method used in the same series.

Note: If end time exceeds the programmed analysis time, this end time overrules the analysis time. You can program events after the end time, but these events are not carried out during a run.

3.8.13 The Method Menu Item < MIX >

If the **MIX** option is enabled in the System menu, the soft function key < MIX > will be presented.

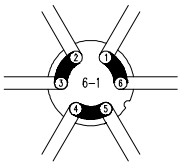
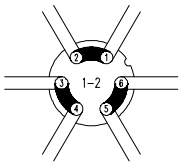
< MIX >	This soft function key is used to access a series of commands to program a method for:
(if enabled in System Menu, Usage Menu)	• pre-injection sample handling (e.g. pre-column derivatization) • dilution • adding of internal standard
	Up to nine mix methods can be programmed. A total of 240 steps can be included in the 9 mix methods and the user program. Assign a number to the mix method.
	When the Mix Menu is presented:
< EDIT >	Press to edit an existing step or a new step for a new mix method.
< INSERT >	Press to insert a new step in an existing method before the displayed step.
< DELETE >	Press to delete the displayed step.
	Note: "End of mix method" means that the mix method is empty; if an existing mix method is selected, the first line of the mix method is displayed. Scroll through the steps of the existing method with the cursor keys and use the soft function keys to enter changes in an existing method.
	The following types of steps can be programmed for a mix method:
< ASPIRATE >	Aspirates a programmed volume (sample, air, destination, reagent A-D). Speed of syringe can be selected from 1-9. (TABLE 3-1). The indicated height (H) is the distance of the needle point to the plate holder (default: 2 mm).The maximum amount which can be aspirated is the total volume of the syringe.
< DISPENSE >	Dispenses (sample, waste, destination, reagent A-D) a programmed volume from the buffer tubing. Speed of the syringe can be selected from 1 - 9 (TABLE 3-1).The indicated height (H) indicated is the distance of the needle point to the plate holder (default: 2 mm). It is possible to dispense a larger volume than the volume aspirated in previous actions. The aspirated amount will be complemented with liquid from the wash solvent bottle to total the programmed dispense volume.
...	

< WAIT >	Defines a pause (H:MM:SS, maximum of 9 hours, 59 minutes and 59 seconds). **Note: During the pause, the needle will move to the home position (if the previous step is an aspirate or dispense action). If you want the needle to stay in the same position, an aspirate or dispense step of 0 µL must be programmed at the desired position.**
< REPEAT >	Enter the number of steps that must be repeated and how often they must be repeated.
< WASH >	Enter the volume for needle wash. Buffer is rinsed to waste.

3.8.14 The Method Menu Item < USER PROGRAM >

If the USER PROGRAM option is enabled in the System menu, the soft key < USER PROGRAM > is presented.

< USER PROGRAM >	The user program offers the possibility to program all possible actions required for a sample handling sequence in separate steps.
(if enabled in System Menu, Usage Menu)	**Note: The total number of steps for the user program and all nine mix methods cannot exceed 240. The user program can be protected by a special user program protection code (System Settings, Usage Menu). If no user program has been programmed yet, "end of user program" is displayed. Otherwise, the first line of the programmed method appears.** The following entries are presented for the user program:
< EDIT >	Press to edit an existing step or a new step for a new mix method.
< INSERT >	Press to insert a new step in an existing method before the displayed step.
< DELETE >	Press to delete the displayed step.
	The edit and insert menus offer the following soft function keys:
< ASPIRATE >	Removes a programmed volume from sample well, ambient air, destination vial, wash, or one of the reagent vials into the buffer tubing. The speed and height of the syringe can be entered (TABLE 3-3). The maximum volume that can be aspirated is the total volume of the syringe.
...	

< DISPENSE >	Delivers a programmed volume from the buffer tubing into the sample well, waste, destination vial, wash or one of the reagent vials. Speed and height of syringe can be entered (TABLE 3-3). It is not possible to dispense a larger volume than the total volume aspirated in previous actions.
< SYR_VALVE >	This command is used to control the connections of the syringe to one of its three tubes: < NEEDLE > to connect the syringe to the sample needle < WASH > to connect the syringe to wash solvent bottle < WASTE > to connect the syringe to the waste tubing.
< SYR >	This command is used to control the movements of the syringe. < LOAD > the syringe with the programmed volume < UNLOAD the syringe with the programmed volume < HOME > the volume previously aspirated will be dispensed to the last programmed position, and the syringe will be initialized again.
< WASH >	This command is used to execute a needle wash; the content of the buffer tubing is not rinsed to waste before the start of the wash. The programmed volume of wash solvent is used to wash the needle at the wash position. **Note: The wash position may be contaminated with the contents of the buffer tubing, which may generate cross-contamination. To prevent contamination of the wash position, program a dispense to waste action before programming a wash action.**
< VALVES >	This command is used to program positions of high pressure valves (ISS, injector valve, SSV). The injector valve has two positions: < INJECT > and < LOAD > . The ISS optional valve has positions indicated by 1-6 (left) and 1-2 (right).  
...	

< WAIT >	This command is used to program a pause (max. 9 hours, 59 minutes, 59 seconds). **Note: During the pause, the needle will move to home position (if the previous step is an aspirate or dispense action). If you want the needle to stay in the same position, an aspirate or dispense step of 0 µL must be programmed at the desired position.**
< COMPRES >	This command is used to activate the compressor to put air pressure on a sample. The compressor will stay active until it is switched off (in a next programmed step). The compressor will be automatically switched off at the end of the needle wash routine if a needle wash is used.
< AUX >	This command is used to control the four standard auxiliaries (contact closures). Refer to Appendix E for details.
< WAIT-IN >	This command is used to program a pause in which the FAMOS Well Plate Microautosampler waits for one of the four inputs to become < HIGH > or < LOW > before continuing with the next step. Refer to Appendix E for details.
< PROG-OUT >	This command is used to define two programmable outputs (contact closures). These are similar to the auxiliaries, but only available in the user program. Refer to Appendix E for details.
< CODE >	to program the output to the connector P3 TIMED OUTPUTS. This is a HEX output in the range 0 to 15. Refer to Appendix E for details.
< MARKERS >	the markers normally generated in the FAMOS Well Plate Microautosampler are not active in the user program, but can be programmed in this screen (refer to Appendix E for details). Select marker and status (inject, vial, labeled).
< SSV > (option)	This command is used to define the Solvent Selection Valve (SSV) port position, range 1 to 6.

TABLE 3-3. Syringe Speed

Speed	Syringe				
	25 µL	*100 µL*	*250 µL*	*500 µL*	*1000 µL*
1	12.5	50	125	250	0.5
2 (low)	31.3	125	315	630	1.3
3 (normal)	62.5	250	625	1250	2.5
4 (high)	93.8	375	940	1880	3.8
5	192.5	770	1920	3840	7.7
6	267.5	1070	2675	5335	10.7
7	342.5	1370	3430	6855	13.7
8	436.3	1745	4365	8725	17.5
9	533.8	2135	5335	10670	21.3
	µL/min				mL/min

CAUTION

Caution: The pressure in the buffer tubing will increase during the dispense action. To prevent damage of the buffer tubing, the flow should not exceed the value of 6mL/min for water. A maximum speed of 9 for 25, 100, and 250 µL-syringes, a maximum speed of 6 for a 500 µL syringe and a maximum speed of 4 for 1000 µL syringe should be used. If more viscous liquids are used, the speeds should be reduced.

3.8.15 The Method Menu Item <TEMPLATE>

If the TEMPLATE option is enabled in the System menu, the soft function key <TEMPLATE> is presented.

<TEMPLATE>	Use this key to enter a menu in which the contents of a template can be defined.
(User program instead of methods)	First assign a number to the template, then link the numbers of methods to the template.
	The following items can be entered to fill a template:
<YES>	The complete template is filled with the user program; no other methods can be added.
<NO>	The template can be filled with the following: **mix method number,** **injection method number,** **wash method number,** **timebase method number** A maximum of 24 templates can be programmed

3.9 Series Menu

The Series Menu allows the user to define the run sequence in a series. A maximum of 24 series can be programmed, each series contains information about the methods to be used for a range of wells. This can be a template, a separate method (mix, injection, wash, timebase), or the user program. Information on location of wells, labeled wells or calibration wells is also programmed in a series. Series parameters are described in TABLE 3-4.

Note: The settings entered in the System Menu and the methods defined in the Methods Menu determine which possibilities appear in the Series Menu.

TABLE 3-4. Series parameters

Without templates	With templates
❍ Use user program Yes/No ● Injection method number ● Wash method number ❍Time base methods number ❍ Mix method number *Time base and mix method are only available if enabled in the System Menu*	● Template number
❍ Use calibration wells Yes/No ❍ First calibration well ❍ Last calibration well ❍ No. of samples between calibration *Calibration wells are only available if enabled in the System Menu; not available if Mix Method has been programmed*	
● First sample well ● Last sample well	
Only if a mix method has been programmed, or if used in user program: ❍ First destination well ❍ Vial Reagent-A ❍ Vial Reagent-B ❍ Vial Reagent-C ❍ Vial Reagent-D	
Only if the use of labeled wells has been enabled in the System Menu: ❍ Labeled well no. 1 ❍ Labeled well no. 2 ❍ Labeled well no. 3 ❍ Labeled well no. 4	

● *marked questions are always asked in series,*
❍ *marked questions depend on the used methods and the settings entered in the System Menu.*

After you have entered the required settings in the System Menu and after you have programmed methods to be used for an analytical run, you can press Series to enter the Series Menu. TABLE 3-4 gives an overview of the items you should to define for the Series.

- **With Templates** - if you are going to execute an analytical run by way of a template, you will only be asked to enter the template number and to indicate the location of the first sample well and the last sample well.

- **Without Templates** - if you are going to execute an analytical run without using a template, you will be asked to enter an injection method number and a wash method number, and you will have to indicate the location of the first sample well and last sample well.

If you have enabled use of calibration wells in the System Menu (Usage Menu), you should define whether you will use calibration wells, and indicate the location of the first and last calibration well, and indicate the number of wells between calibration wells (FIGURE 3-6).

However, if you have for example enabled use of a Mix Method in the System Menu (Usage Menu), you will also have to define the location of the First destination well and Reagent vials.

Note: Series are stored in the microautosampler memory for as long as the power is on. As soon as power is switched off, all programmed series will be deleted. It is not possible to leave the Series Menu before all values have been programmed.

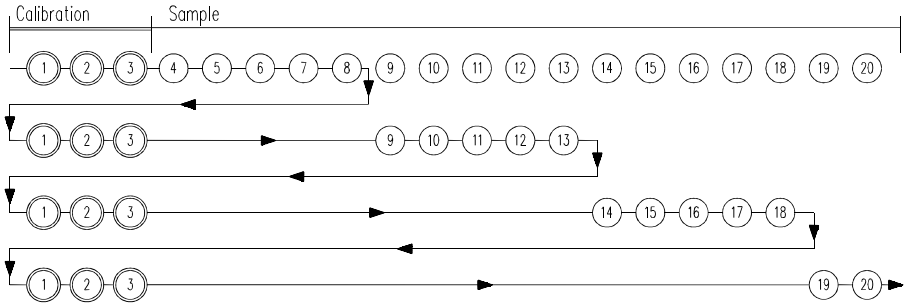


FIGURE 3-6. Injection sequence with 3 calibration wells between every 5 wells

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CHAPTER 4

Programming Examples

4.1 Overview

The commands described in Chapter 3 can be used by the analyst to generate a broad range of programs for the operation of the microautosampler. These commands allow the user to configure the unit to meet the specific needs of the laboratory. A series of blank forms are provided to assist the analyst in programming (Appendix F).

This chapter describes the editing of general system settings and selection of the plate. In addition, a few simple programs that show the general approach to programming (additional programs are presented in Appendix C) are included. While these programs may not meet the specific needs of the analyst, it is likely that they can be used with minor modification (e.g. parameters such as the analysis time and sample volume may need to be changed). An additional rationale for the presentation of these programs is to provide a description of the logical processes that are used in generating a program.

It should be noted that the examples described below could be used with systems that include *UltiMate*™ with the *UltiChrom*™ software program as well as for stand-alone systems.

The following programs are included in this chapter:

- Single 200 nL injection (shows partial loopfill) - Section 4.4.2.
- 100 nL injection from of 3 vials and 5 nL injections from 3 other vials (shows partial loopfill and injections from multiple vials)- Section4.4.3.
- 1 µL injection out of a 5 µL sample in vial 1 using transport liquid. (shows µL pickup) – Section 4.4.4.

Additional examples are provided in Appendix C.

4.2 General System Settings

When the microautosampler is initially installed, a variety of general parameters are set which generally define the configuration of the unit and should be checked. When you are defining a new method, you should check these values to make sure that they are relevant.

To check the general system settings, press the **System** key, then select the soft function keys < MICRO > and < GENERAL > as appropriate and press the **E** key on a sequential basis to view each selection.

Use the **E** key to go through the general system settings. A set of reasonable values is presented in TABLE 4-1.

Note: The E key is used to indicate that the user accepts the indicated value for a parameter and he/she wants to access the next parameter field. For the sake of brevity, it will be understood that the E key must be pressed when a parameter is viewed/edited.

TABLE 4-1. Typical General Parameter Settings

< GENERAL > Parameter	Setting
Volume of the installed injection loop	5 µL
Volume of the tubing 'needle – valve'	2.4 µL
Syringe speed	low
Scale factor	0.2
Sample needle height	04 mm
Skip missing vials	yes
Air segment	no
Headspace pressure	yes
Time base display	HH:MM:SS or H:MM:mm
Key click	on
Error beep	on
Alarm buzzer	on

To go back to the Ready menu, press **Escape** twice.

Note: Adjust the needle height to the desired depth of insertion. Use 4mm ([04]) for the enclosed 250 µL polypropylene tapered vials.

4.3 Installing the Plate

To install the plate:

a) Insert the 48 vial holder (FIGURE 4-1). It will be necessary to use the <EXCHANGE> soft key to move the plate holder to the left to access the plate holder.

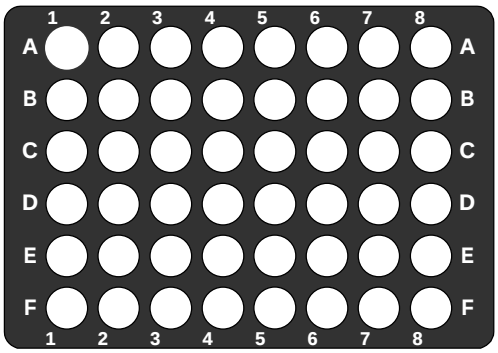

FIGURE 4-1. 48 Position Well Plate

b) Press the <PLATE HOME> soft key to move the plate holder back into operation position and place a sample vial in position A1.

To check the plates setting:

a) Press the **System** key and then select the soft function keys <MICRO> and <PLATES>).

b) Select <48 vials>.

c) Select Process Vials <IN ROWS>.

To go back to the Ready Menu, press **Escape** twice.

4.4 Sample Applications

4.4.1 Conventions

The following typographical conventions are used:

Initial Capitals	represents the names of the menus (e.g. Ready Menu).
<CAPTALS>	represents the soft function key (e.g. <INJECTION>).
bold type	represents the names of the function keys (e.g. **E**).
[01.23]	indicates that a numerical value has to be entered

4.4.2 Example 1: Injecting a Single 200 nL Sample from 1 vial

This example describes an application in which a single injection is made from a single vial in position A1.

a) Place a sample in position A1.

b) Program the Injection Method by entering the parameters indicated in TABLE 4-2.

TABLE 4-2. Programming the Injection Method

Press keys	Description
Methods	to enter the Methods Menu
<INJECTION> [01] **E**	to define the number of the method
<PARTIAL> **E**	to select partial loop fill
[25.00] **E**	to set analysis time to 25 min
[5.0] **E**	to set the flush volume to 5µL
[01] **E**	to set the number of injections/vial to 1
[02] **E**	to set injection volume to 0.2 µL
<STD> **E**	to select the standard injection control
Escape Escape	to return to the Ready Menu

c) Program the Series by entering the parameters indicated in TABLE 4-3.

TABLE 4-3. Programming the Series:

Press keys	Description
Series	to enter the Series Menu
[01] **E**	to define the Series number
[01] **E**	to select the injection method number
CL E	to enter <NONE> for wash method
<ROW A> [01] **E**	to define location of the first sample vial
<ROW A> [01] **E**	to define location of the last sample vial
Escape	to return to the Ready Menu

d) Run the series by entering the parameters indicated in TABLE 4-4 and pressing < START > .

TABLE 4-4. Running the Series

Press keys	Description
Start/Stop	to start the FAMOS Well Plate Microautosampler
[01] **E**	to start at series 01
[01] **E**	to stop after series 01
< START >	to start execution of the series.

At the end of the defined analysis time the **Ready Menu** will appear again to indicate that the FAMOS Well Plate Microautosampler is ready for the next run.

4.4.3 Example 2: Injecting a Single 100 nL sample from 3 vials and Injecting 5 µL samples from 3 other vials

This example describes an application in which a single injection is made from each of three vials and a single injection with a different volume made from three additional vials.

a) Place the appropriate vials in positions A1-A3 (for the 100 nL Injections) and the appropriate vials in positions C1-C3 (for the 5 µL injections).

b) Program the injection method for the 100 nL injections by entering the parameters indicated in TABLE 4-5.

TABLE 4-5. Programming the Injection Method for 100 nL Injections

Press keys	Description
Methods	to enter the Methods Menu
< INJECTION > [01] **E**	to define 1 for the first method
< PARTIAL > **E**	to select partial loop fill
[25.00] **E**	to set analysis time to 25 min
[5.0] **E**	to set the flush volume to 5µL
[01] **E**	to set the number of injections/vial to 1
[01] **E**	to set injection volume to 0.1 µL
< STD > **E**	to select the standard injection control
Escape	to return to the Methods Menu

c) Program the injection method for the 5 µL injections by entering the parameters indicated in TABLE 4-6.

TABLE 4-6. Programming the Injection Method for 5 µL Injections

Press keys	Description
< INJECTION > [02] **E**	to define 2 for the second method
< FLUSHED > **E**	to select flushed loop fill
[25.00] **E**	to set analysis time to 25 min
[5.0] **E**	to set the flush volume to 5µL
[01] **E**	to set the number of injections/vial to 1
< STD > **E**	to select the standard injection control
Escape Escape	to return to the Ready Menu

d) Program the series by entering the parameters indicated in TABLE 4-7.

TABLE 4-7. Programming the Series

Press keys	Description
Series	to enter the Series Menu
[01] **E**	to define the Series number
[01] **E**	to select the injection method number
CL E	to enter <NONE> for wash method
<ROW A> [01] **E**	to define location A1 as first sample vial
<ROW A> [03] **E**	to define location A3 as last sample vial
Series	to enter the Series Menu
[02] **E**	to define the next Series number
[02] **E**	to select the next injection method number
CL E	to enter <NONE> for wash method
<ROW C> [01] **E**	to define location C1 as first sample vial
<ROW C> [03] **E**	to define location C3 as last sample vial
Escape	to return to the Ready Menu

e) Run the series by entering the parameters indicated in TABLE 4-8 and pressing <START>.

TABLE 4-8. Running the Series

Press keys	Description
Start/Stop	to start the FAMOS Well Plate Autosampler
[01] **E**	to start at series 01
[02] **E**	to stop after series 02
<START>	to start execution of the series.

At the end of the defined analysis time, the Ready Menu will appear again to indicate that the FAMOS Well Plate Microautosampler is ready for the following next run.

4.4.4 Example 3: 1µL injection out of a 5 µL sample in vial A1 using the transport liquid (µL pick-up)

This example describes an application in which a single injection is made from a vial position A1 using transport liquid for pickup. The µL pick-up routine allows for an injection to be made from small sample volumes with essentially no sample loss.

To perform this operation

a) Place a 250 µL tapered sample vial in vial position A1 and one of the 10 mL capped vials in transport vial position 1 (leftmost position in the rack) as shown in FIGURE 4-2 (the transport liquid should have a lower strength than the mobile phase).

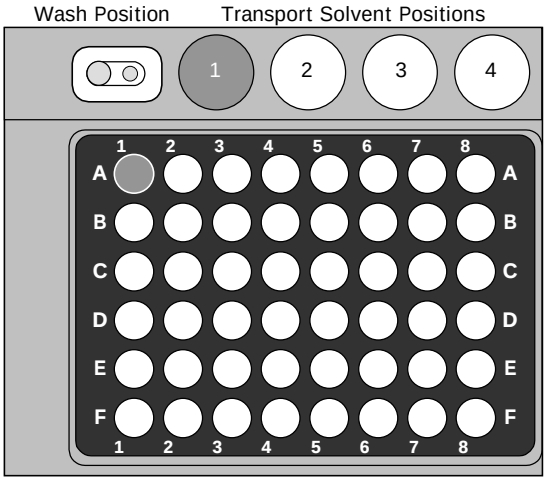

FIGURE 4-2. 48 Well Plate Holder and Location of Transport Liquid/Sample Vials

b) Install a 10µL sample loop.

c) Set the loop volume to 10 µL and verify that the right needle height (4 mm) has been set so that the needle can reach the bottom of the tapered sample vial (see Section 4.2).

d) Identify the position of the transport liquid vial by entering the parameters indicated in TABLE 4-9.

TABLE 4-9. Identifying the Location of the Transport Vial

Press keys	Description
System	to enter the system settings
< MICRO > **E**	to select micro mode
< PLATES > **E**	to select the right plate version
< 48 > **E**	
< IN ROWS >	to process the well in rows
[01] **E**	to define the location of the first transport liquid vial
[01] **E**	to define the location of the last transport liquid vial
Escape Escape	to return to the Ready Menu

e) Program the Injection Method by entering the parameters indicated in TABLE 4-10.

TABLE 4-10. Programming the Injection Method

Press keys	Description
Methods	to enter the Methods Menu
< INJECTION > [01] **E**	program injection method number 1
< PICK UP > **E**	to select partial loopfill injection method
[25.00] **E**	to define an analysis time of 25 minutes
[1] **E**	to define the number of injections/vial
[1.00] **E**	to set the injection volume to 1.00 µL
Escape Escape	to return to the Ready Menu

f) Define the Series by entering the parameters indicated in TABLE 4-11.

TABLE 4-11. Programming the Series

Press keys	Description
Series	to enter the Series Menu
[01] **E**	to define the Series number
[01] **E**	to select the injection method number
CL E	to enter < NONE > for wash method
< ROW A > [01] **E**	to define location A1 as first sample vial
< ROW A > [01] **E**	to define location A1 as last sample vial
Escape	to return to the Ready Menu

g) Run the series by entering the parameters indicated in TABLE 4-12 and pressing < START > .

TABLE 4-12. Running the Series

Press keys	Description
Start/Stop	to start the FAMOS Well Plate Microautosampler
[01] **E**	to start at series 01
[01] **E**	to stop after series 01
< START >	to start execution of the series.

At the end of the defined analysis time the Ready Menu will appear again to indicate that the FAMOS Well Plate Microautosampler is ready for the following next run.

CHAPTER 5

Testing the Microautosampler

5.1 Overview

This chapter describes a series of activities that can be used to check the operation of the system and to verify that your FAMOS™ Well Plate Microautosampler is operating in an acceptable manner.

This chapter includes:

- Establishing a Test Protocol (Section 5.2)
- Running the Test Protocol (Section 5.3)

5.2 Establishing a Test Protocol

This section describes the parameters for the test protocol that will be performed in Section 5.3. A detailed discussion of how the parameters are accessed and edited is presented in Chapter 3.

a) Verify that the microautosampler is configured as listed in TABLE 5-1 and set the values in TABLE 5-1 for the General Parameters.

TABLE 5-1. General Parameters

Description	Value
Loop volume	5 µL
Needle tube volume	2.5 µL
Syringe volume	25 µL
Dispenser speed	Low/0.2
Sample needle height	2 mm
Skip missing vial	YES
Air segment	NO
Head space pressure	YES

b) Set the well plate type you are using.

c) Program the Partial Loopfill method presented in TABLE 5-2.

TABLE 5-2. Method Parameters

Description	Value
Injection method number	1
Analyze time	1:00 min
Flush volume	5 µL
Injections/vial	9
Injection volume	0.10 µL
Low dispersion mode	off

d) Program the Series presented in TABLE 5-3.

TABLE 5-3. Series Parameters

Description	Value
Series number	1
Injection method number	1
Wash method	None
First vial	ROW A01
Last vial	ROW A01

5.3 The Test Protocol

5.3.1 The Analytical System used for the Test Protocol

In this test protocol, a LC Packings *UltiMate*™ Micropump (with a CAP-300 calibrator) and an *UltiMate* UV Detector, set at 254 nm are used (FIGURE 5-1). The *UltiChrom*™ software is used to control the system and acquire/present the data. A 1 m fused silica tube (75 μm I.D.) should be placed between the autosampler and the detector to create backpressure.

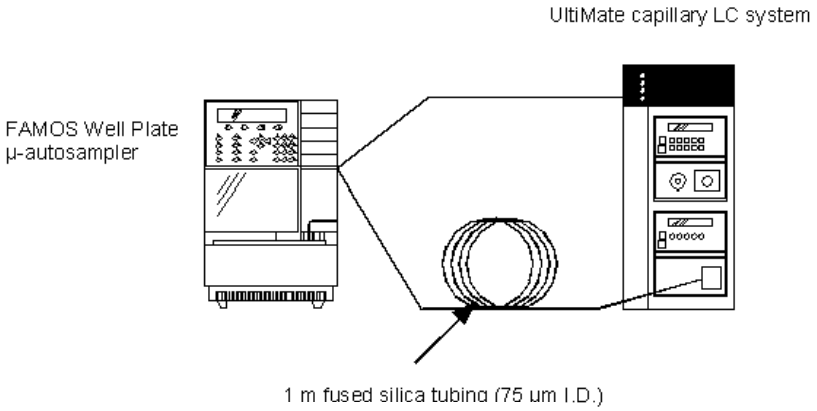

FIGURE 5-1. Analytical System for Test Protocol

If an *UltiMate* system is not available, any HPLC system with reproducible microflow capabilities can be used (e.g. a conventional HPLC pump with an Acurate™ flow splitter).

Note: The entire flow path from the syringe to the needle should be carefully inspected before the test protocol is performed. This test should not be performed if the ambient temperature is below 18°C.

5.3.2 The Test Protocol

Prepare the FAMOS Well Plate Microautosampler as follows:

- Place a closed vial at position A01, the sample is Uracil in distilled water ~0.1 mg/mL The eluent is distilled water.
- Fill the wash solvent bottle with 80% H_2O / 20% isopropanol.
- Program a partial loop fill method using the settings in Section 5.2.

Obtain the peak area for each peak and determine the value for σ, as well as the RSD%, reproducibility, which should be ≤1.0% using the equations presented below.

$$\overline{Peak\ area} = \frac{\sum Peak\ area}{n}$$

$$\sigma_{n-1} = \sqrt{\frac{\sum \left(Peak\ area - \overline{Peak\ area}\right)^2}{n-1}}$$

$$RSD\% = \frac{\sigma_{n-1}}{\overline{Peak\ area}} \times 100\%$$

A typical chromatogram from the test is presented in FIGURE 5-2 and sample data is presented in TABLE 5-4.

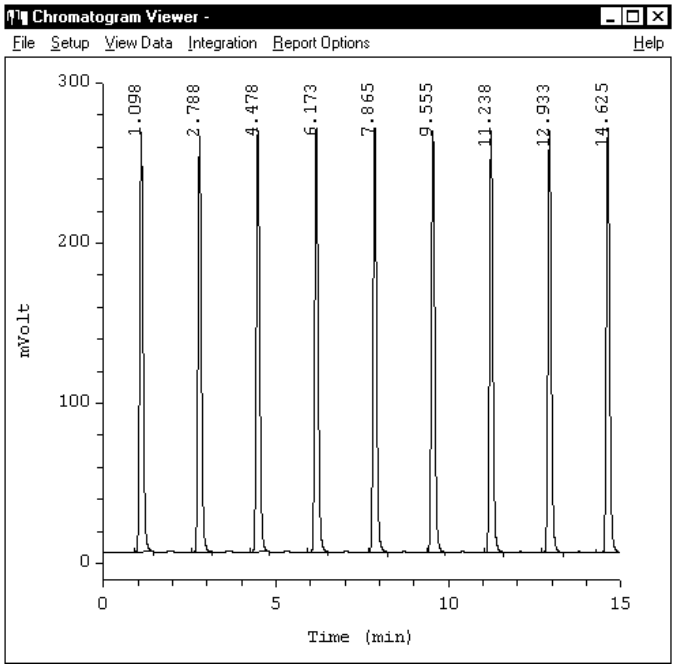

FIGURE 5-2. Typical Sample Chromatogram for the reproducibility test

TABLE 5-4. Sample Peak Height Data

Peak number	Height
1	216.8
2	259.6
3	262.8
4	264.4
5	265.0
6	263.0
7	263.4
8	262.9
9	265.3

For this example, the results are:

RSD% = 0.66 %

The specified value is an RSD% < 1%.

If the reproducibility is not within the specification:

a) Check for any air bubbles in the tubing/syringe.

b) Check needle and tube connections between needle and injection valve for dead volume or blockage.

c) Review the Troubleshooting Section (Section 6.3).

Once you have remedied the problem, repeat the performance test.

5.4 Checking System Components

5.4.1 Testing the Accuracy of Delivery of the Syringe

To determine the accuracy of delivery of the syringe, dispense a volume of 25 µL of water from a sample vial to a destination vial, program the following Mix Method (TABLE 5-5) and Series (TABLE 5-6).

TABLE 5-5. Mix Method 1

Step	Action	Speed	Height
1	Aspirate 25 µL Sample	2	05
2	Dispense 25 µL to Destination	3	03
3	End of mix method		

TABLE 5-6. Series 1

Description	Value
Series Number	1
Injection method	None
Wash method	None
Mix method	1
First well	ROW A 01
Last well	ROW A 01
First destination vial	ROW A 02
Reagent A vial	1

To determine the accuracy of delivery of the syringe:

a) Weigh the destination vial before and after the Run.

b) The difference is the aspirated volume

The specified variation is ± 2%.

5.4.2 Testing the Loop Volume

To determine the volume of the injection loop

a) Disconnect the loop from the injection valve.

b) Remove all liquids from the loop with air.

c) Weight the empty loop on an analytical balance.

d) Fill the loop with minimal 2 times its volume of water.

e) Weight the filled loop again.

The difference in the weight of the loop, divided by the specific weight of water (1 g/mL) gives the calibrated volume of the loop.

The specified variation is ± 10%.

5.4.3 Testing the Tray Cooling Option

To test the tray cooling option (if installed):

a) Remove the plate holder.

b) Place a thermocouple in the middle of the cooling plate (flat surface underneath the wash solvent bottle, item 3, FIGURE 2-5), making sure that a good contact is made.

c) Switch on the cooling and program a setpoint of 10°C.

d) Wait minimal 15 minutes for equilibration of the FAMOS Well Plate Microautosampler.

e) Read out the temperature of the thermocouple.

The value must be in a range of ± 2°C of the programmed setpoint.

Note: When the cooling option is used, the temperature of the sample inside the sample vial may be slightly different than the temperature set via the command, due to a variety of effects (e.g. the heat transfer characteristics of the vial walls). If it is necessary to precisely set the temperature of the sample, we recommend that you determine the temperature inside the vial at various temperature setpoints and set the cooling option so that the desired internal temperature is attained.

CHAPTER 6

Maintenance and Troubleshooting

6.1 Overview

This chapter provides information to assist in optimizing the performance of the FAMOS™ Well Plate Microautosampler and maintaining it in your laboratory. It includes the following material:

- **Maintenance** - describes a series of activities that should be performed on a periodic basis to optimize the performance of the system and minimize down time (Section 6.2).
- **Replacing Components** – provides directions for replacing components due to wear or to re-configure the system (e.g. changing the syringe) to meet the requirements of a different analytical procedure (Section 6.3).
- **Troubleshooting** - discusses a series of activities that should be used to determine the cause of a problem. (Section 6.4).
- **Error Codes -** lists and describes various messages that are presented on the display to indicate a fault with the system (Section 6.5).
- **Spare Parts/Replacement Parts Lists** – Presents a listing of components that are used to maintain the unit or to change the configuration (Section 6.6).

6.2 Maintenance

Maintenance refers to a variety of activities that should be performed on a routine basis to optimize performance of the system. Many routine maintenance activities can be readily performed by the user.

In some cases (e.g. replacement of critical components), we recommend that a factory trained service engineer should be called to perform the operation. This will ensure optimal long term performance and maximum uptime. LC Packings provides a broad range of service support activities to ensure that the FAMOS Well Plate Microautosampler is functioning in a suitable manner. These activities can be customized to meet the specific needs of the customer. For further information, please contact your local LC Packings office or representative.

TABLE 6-1. Recommended Maintenance Schedule

Frequency	Operation
Every Day	Before operating, check for any air bubbles in the fluidic lines and degas the wash solvent.
	Check that there are no leaks of the fluidics connections.
	Check that salts are not deposited by the fluidics joints.
	When using buffer solutions, flush the system thoroughly after use with a solvent that contains does not contain buffers/salts.
Every 3 months	Inspect the condition of all tubing (cracks, nicks, cuts, clogging).
Every year	Replace:
	Rotor Seal
	Sample Needle
	Prepuncturing Needle
	Syringe tip
	Connections on Injection Valve and Syringe
	Check:
	Stator

Note: The frequency of the various activities described above is a good starting point. As the user gains experience with the system, it will be found that some activities can be done less frequently and other need to be done more frequently. The frequency is dependent on a number of factors including the nature of the sample and the mobile phase.

6.3 Replacing Major Components

A variety of components on the FAMOS Well Plate Microautosampler can be readily changed by the user as required to ensure that the instrument is maintained in optimal condition. In some circumstances the analyst may want to change a component, as an example, four different needles are available (see Section 6.6.2) and it is possible that the application would be best served with a silica coated needle if the sample could interact with the stainless steel needle.

6.3.1 Replacing the Syringe

The FAMOS Well Plate Microautosampler is supplied with a 25 µL syringe (standard configuration). In addition, a 100, 250, 500 or 1000 µL syringe is available.

To replace the syringe:

a) Press the <SYR END> soft function key in the Ready Menu to move the syringe to the end position, and then lift the cover.

b) Unscrew the top of the syringe (turn clockwise). Pull the syringe down until it releases from the syringe valve. Then pull the syringe towards you and remove it (FIGURE 6-1).

c) Unscrew the Luer Lock adapter (100, 250, 500 or 1000 µL syringe only).

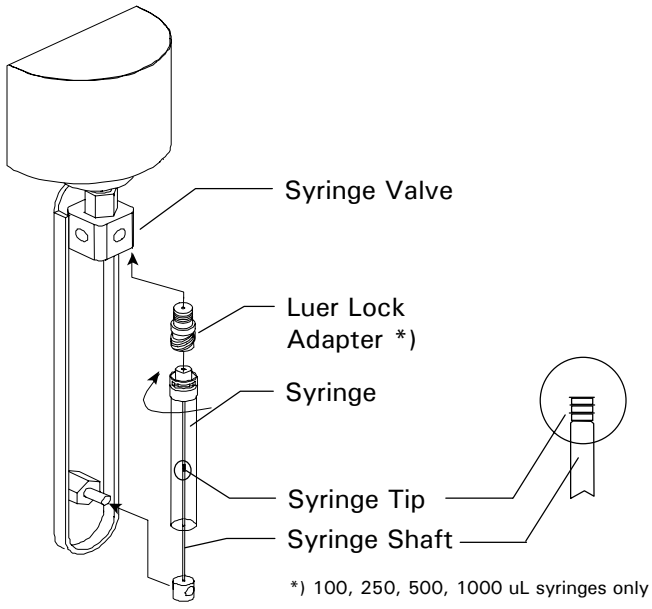

FIGURE 6-1. Replacing the syringe

d) Attach the Luer Lock adapter to the new syringe (100, 250, 500 or 1000 µL syringe only).

Note: The 100, 250, 500 and 1000 µL syringes require the Luer Lock adapter (P/N 160137).

e) Fill the new syringe with wash solvent and make sure that all air bubbles are removed from the syringe. Connect the bottom of the filled syringe to the FAMOS Well Plate Microautosampler. Screw the top of the filled syringe to microautosampler (counter clockwise).

f) Lower the cover. Press the <SYR HOME> soft function key to remove air from the syringe. The syringe moves to the home position and the content is dispensed to waste.

Caution: Before continuing, check if the correct syringe is selected in the System Menu and the correct buffer tubing is installed (refer to Section 2.3). An incorrect setting may lead to damage to the system.

g) Select the <WASH>soft key in the Ready Menu to execute a standard wash routine. All tubing connected to the syringe valve is filled and rinsed.

6.3.2 Replacing the Syringe Tip

To replace the tip of the syringe:

a) Remove the syringe as described in Section 6.3.1, items a-c.

b) Remove the syringe tip.

c) Place a new tip on a flat surface (e.g. workbench), making certain that attachment point is facing up.

d) Gently push the syringe shaft in the new tip.

Caution: If you are installing a 25 µL syringe tip, be extremely careful as the shaft is very thin and can be easily bent.

e) Carefully insert the shaft in the syringe body.

f) Re-install and flush the syringe as described in Section 6.3.1 item d) to g).

g) Check for any leakage.

6.3.3 Needle Assembly

The sampling needle consists of two parts:

- **Sample needle:** placed inside the hollow prepuncturing needle; used for the actual transport of sample. Different types of needles can be used (see Section 6.6.2). If a needle with different diameter is used, a different air outlet nut (item 6, FIGURE 6-2) must be used that matches the injection needle. The fused silica needle (P/N 160116) is the default needle.

- **Prepuncturing needle:** a hollow needle used for puncturing of the septum, capmat or sealer; also used to put headspace pressure on the sample (approximately 0.5 bar).

Note: Most commercially available sealers or capmats cannot be used in combination with headspace pressure. We recommend that headspace pressure is switched off in those cases (System Menu, General Menu).

A schematic diagram of the needle assembly is shown in FIGURE 6-2 and a list of the optional needles is presented in TABLE 6-2 and TABLE 6-3.

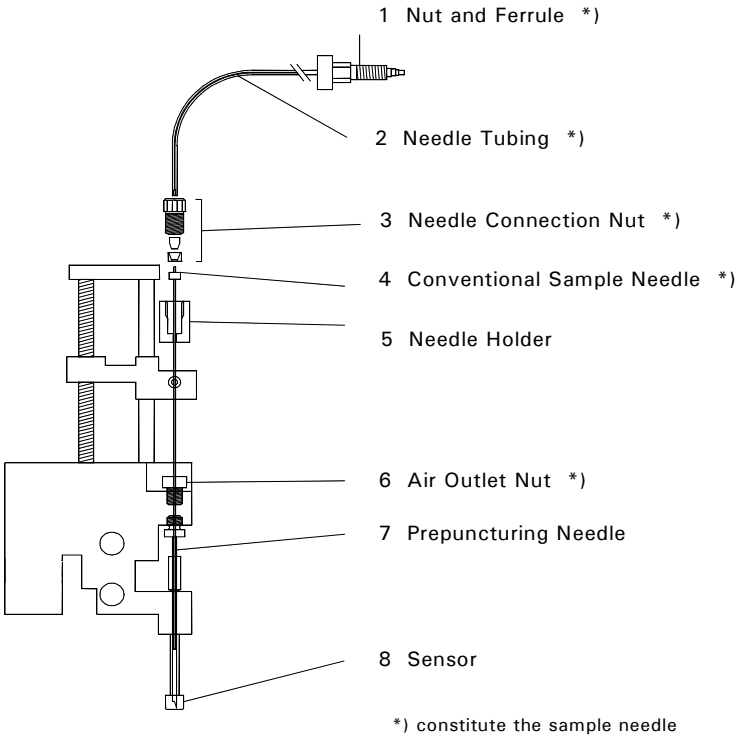

FIGURE 6-2. Needle Assembly

TABLE 6-2. Optional Needles

Needle Type	Description
Fused silica needle	needle with small inner diameter for small injection volumes
Stainless steel needle	sample needle with large inner diameter for viscous samples, or in case large volumes are loaded in the loop
Extended needles	needles for switching valve placed in the side panel of the FAMOS Well Plate Microautosampler

Refer to Section 6.6 for an overview of options available for the FAMOS Well Plate Microautosampler

To replace the present needle:

a) Loosen the needle connection nut (item 3, FIGURE 6-2).

b) Loosen the VICI-Valco and ferrule nut (item 1, FIGURE 6-2).

c) Carefully pull out sample needle and tubing.

d) Remove the air outlet nut and replace it by a new one.

e) Insert a new sample needle and tube through the needle holder and tighten the nut.

f) Connect the other end of the tube to port 4 of the VICI-Valco injection valve using a VICI-Valco nut and ferrule (item 1, FIGURE 6-2). Do not overtighten (to prevent block of tubing).

g) Lower the cover of the FAMOS Well Plate Microautosampler.

h) Check sample needle height (default height: 2 mm from plate). If necessary, adjust the value in the System Menu (General Menu; refer to Chapter 3).

i) Select the <WASH> soft key in the Ready Menu to clean the new sample needle.

To install a different needle:

a) Remove needle as described above.

b) Remove the standard air outlet nut (Item 6, FIGURE 2-3, item 6) and replace it by the nut supplied with the optional sample needle.

c) Install the optional sample needle as described in Section 6.4.3.

d) Adjust settings in System Menu (General Menu) to the volume of the new needle tubing (see section *User Interface*).

TABLE 6-3. Characteristics of Needles for FAMOS Well Plate Microautosampler

Needle				**Tubing**			**Total**
Part Number	O.D. (mm)	I.D. (mm)	Length (mm)	O.D. (mm)	I.D. (mm)	Length (mm)	Volume
Fused Silica Needles							
160116 / 162000 a)	0.280	0.10	300	one piece			2.4 µL
160117 b)	0.375	0.15	585				10.3 µL
Stainless Steel Needles							
160119	0.64	0.25	135	1.6	0.25	140	15 µL
contact LC Packings b)	0.64	0.25	135	1.6	0.25	420	30 µL

a) inert version, b) extra long version
Refer to Section 6.6 for an overview of options available for the FAMOS Well Plate Microautosampler

Note: When the injection valve is mounted on the right side, e.g. using the FAMOS Well Plate Microautosampler in combination with the THERMOS™, the sample needle should be an extra long version.

6.3.4 Prepuncturing Needle

To replace the prepuncturing needle:

a) Remove sample needle (Section 6.3.3).

b) Unscrew the prepuncturing needle.

c) Install a new needle using a new seal.

d) Reinstall the sample needle (Section 6.3.3).

6.3.5 Combination Syringe, Sample Loop and Buffer Tubing

This section will indicate the standard configuration of the FAMOS Well Plate Microautosampler and appropriate configuration for three commonly selected modes of using the microautosampler (a detailed discussion of the injection principles is presented in Appendix A):

- Injection volumes smaller than 1 µL
- Injection volumes up to twice the standard (conventional mode)
- For volumes larger than 200 µL

A) Standard Configuration

The standard configuration for the FAMOS Well Plate Microautosampler includes a 2.4 µL Fused Silica Needle, a 25 µL syringe; a 50 µL buffer tubing and a 10 µL sample loop. The injection volume ranges shown in TABLE 6-4 are available for the various injection modes while TABLE 6-5 presents the maximum injection volumes for the various injection modes. Five sizes of syringes can be used in the FAMOS Well Plate Microautosampler, 25, 100, 250, 500 and 1000 µL.

TABLE 6-4. Injection Ranges

Injection Mode	Injection Volume Range	Maximal Injection Volume
Full loop	10 µL	loop volume (injection volume fixed by loop size)
Partial loopfill	0.01 - 5 µL	50% of loop volume
µL pick-up	0.01 – 1.4 µL	(loop volume - 3 x needle tubing volume) / 2

The characteristics of each injection mode is described below:

- **Full loop injection** - gives maximum reproducibility (RSD < 0.4%).
- **Partial loopfill** - gives maximum accuracy (depends on syringe accuracy) and reproducibility better than 0.6% RSD for injection volumes > 1 µL.
- **µL Pick-up** - offers zero sample loss, maximum accuracy (same as partial loopfill) and RSD better than 1.5% for injection volumes ≥ 1 µL.

A discussion of the appropriate modes for three common scenarios is presented below.

B) Injection Volumes Smaller than 1 µL

Partial loopfill: For maximum reproducibility and accuracy a 25 µL syringe and a 1 µL sample loop should be used.

µL Pick-up: For optimum accuracy and reproducibility a 25 µL syringe and a 10 µL sample loop should be used. The sample plug is transported into the loop, preceded by a plug of transport liquid that has a volume of 2.5 times the programmed needle tubing volume.

C) Injection Volumes up to Twice the Standard

With a 100 µL syringe, a stainless steel needle with tubing (15 µL) and 500 µL buffer and a 50 µL sample loop, the maximum injection volumes are shown in TABLE 6-5.

TABLE 6-5. Maximum Injection Volume

Injection Mode	Maximal Injection Volume
Full loop	50 µL (sample loss equals 130 µL; two loop volumes overfill; 30 µL pre-flush)
Partial loopfill	25 µL
µL pick- up	2.5 µL

D) For Volumes Larger than 200 µL

With the 2000 µL buffer tubing, use the appropriate sample loop size and the appropriate syringe. The syringe volume should be at least 2 x injection volume.

Note: Injection volumes larger than 500 µL are possible, but the sample may contaminate the syringe. Make certain that you have programmed a sufficient wash after use

6.3.6 Replacing the Main Fuses and Setting the Operation Voltage

DANGER

Danger: Disconnect the instrument from the electrical supplies before inspecting/changing the fuse.

To change the Fuses:

a) Pull out the fuse holder (FIGURE 6-3, item 1).

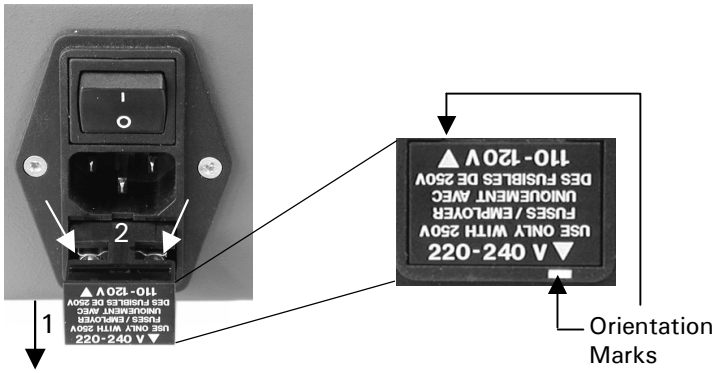

FIGURE 6-3. The Fuse Compartment

b) Replace the blown fuse(s) by fuse(s) of the same rating:

115 V (AC) ± 10%:
two 5 AT fuses (slow, ¼" x 1¼", UL/CSA)
230 V (AC) ± 10%:
two 2.5 AT fuses (slow, 5 x 20 mm, IEC127)
(The fuses used are UL-listed and CSA-certified)

c) Re-install the fuse holder.

Caution: Make certain that the fuse holder is inserted in the correct position so that the appropriate voltage setting is indicated (FIGURE 6-3 indicates the configuration for 220-240 V).

6.4 Troubleshooting

Troubleshooting refers to the determination of the cause of a problem. Since the FAMOS Well Plate Microautosampler is incorporated into an HPLC system, the first step to determine if the problem is due to the microautosampler is to remove the unit from the system, install a manual injector and then perform an injection and compare the results from the two runs. If the results are fine, the problem is due to the autosampler.

Analytical problems also might be caused by external influences, like temperature and/or light sensitive samples. For this reason it is important to be sure the application was running without problems before and nothing has been changed.

A chart outlining the most common faults and the remedies is presented in FIGURE 6-4.

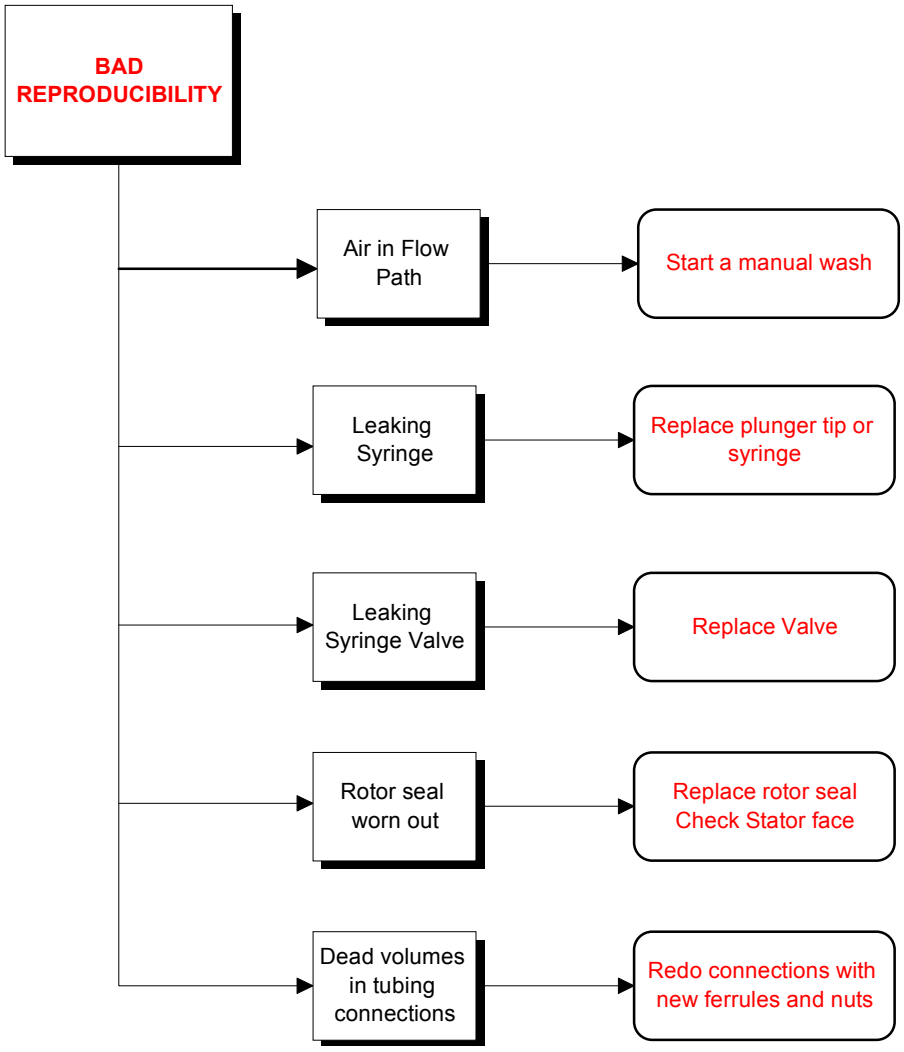

FIGURE 6-4. Troubleshooting the Microautosampler

Note: It is important to note that analytical problems might be caused by external influences, such as the temperature and or the analysis of light sensitive samples. When troubleshooting, make certain that the application was running in an acceptable fashion before the problems were observed and that nothing has been changed in the application.

6.5 Error Codes of the Microautosampler

The FAMOS Well Plate Microautosampler will display an error message if the user tries to enter invalid data and information on the allowed range will be displayed. In addition, several sensors are installed to check for possible mechanical failures and protect the instrument from severe damage.

If a fault or mechanical failure is observed during the operation of the FAMOS Well Plate Microautosampler, an error code will be presented on the display. When a message is indicated, press the **Start**/**Stop** key twice to remove the message and correct the fault.

The error codes are indicated in TABLE 6-6 to TABLE 6-11.

TABLE 6-6. Error Codes for Injection Valve and ISS Unit

Error Code	Probable Cause	Solution
ERROR 11	Injection valve is not in a valid position.	Contact LC Packings.
ERROR 12	The injection valve did not switch within 1.5 seconds.	Contact LC Packings.
ERROR 13	The switching time of the injection valve exceeds 500 msec.	Contact LC Packings.
ERROR 14	ISS valve is not in a valid position.	Contact LC Packings.
ERROR 15	The ISS valve did not switch within 1.5 seconds.	Contact LC Packings.

TABLE 6-7. Error Codes for Syringe Dispenser Unit

Error Code	Probable Cause	Solution
ERROR 21	The syringe valve did not switch.	Contact LC Packings.
ERROR 22	The syringe did not reach home position in time.	Contact LC Packings.
ERROR 23	The syringe spindle did not make the correct number of rotations.	Contact LC Packings.
ERROR 24	The spindle does not rotate.	Contact LC Packings.
ERROR 25	The syringe valve did not find a valid position.	Contact LC Packings.

TABLE 6-8. Error Codes for Plate and Plate Holder

Error Code	Probable Cause	Solution
ERROR 59	Missing plate.	Check for Plate.
ERROR 90	Plate home time-out, plate did not reach home position (home error).	Reinstall Plate Holder.
ERROR 91	Plate did not reach or leave home position during run.	Reinstall Plate Holder.
ERROR 92	Plate holder missing.	Check/reinstall Plate Holder.
ERROR 93	Plate holder movement is blocked.	Check for blockage.

TABLE 6-9. Error Codes for Injection needle unit

Error Code	Probable Cause	Solution
ERROR 30	The sample needle arm did not reach or leave home position (vertical).	Contact LC Packings.
ERROR 31	The sample needle arm is in an invalid horizontal position while moving down.	Contact LC Packings.
ERROR 32	The sample needle arm did not reach or leave destination within a certain time (horizontal).	Contact LC Packings.
ERROR 34	Sample needle arm not in vertical home position while moving horizontally.	Contact LC Packings.
ERROR 39	Vial sensor sticks	Contact LC Packings.
ERROR 40	The sample needle spindle does not rotate correctly.	Contact LC Packings.
ERROR 41	The sample needle did not reach or leave home position.	Contact LC Packings.
ERROR 42	The sample needle is not at home position.	Contact LC Packings.
ERROR 53	The sample needle arm is not in the home position while moving the plate.	Contact LC Packings.

TABLE 6-10. Error Codes for Vials

Error Code	Probable Cause	Solution
ERROR 60	Missing vial. Only available when Skip Missing Vial is set to NO in the System Settings and during the execution of the Mix of a sample on 48-vial plate.	Check/install the vial.
ERROR 62	Missing transport vial.	Check/install the vial.
ERROR 64	Missing vial for reagent A.	Check/install the vial.
ERROR 65	Missing vial for reagent B.	Check/install the vial.
ERROR 66	Missing vial for reagent C.	Check/install the vial.
ERROR 67	Missing vial for reagent D.	Check/install the vial.
ERROR 68	Missing destination vial.	Check/install the vial.
ERROR 69	Not enough transport liquid available due to missing transport vials.	Check/install the vial.

TABLE 6-11. Error Codes for Electronics

Error Code	Probable Cause	Solution
ERROR 71	Flex PCB of the sample needle is not properly connected.	Contact LC Packings
ERROR 72	Invalid configuration of the FAMOS Well Plate Microautosampler, PCB missing.	Contact LC Packings
ERROR 73	Current limit of the external I/O exceeded.	Contact LC Packings
ERROR 75	Error occurred during initialization, the FAMOS Well Plate Microautosampler cannot start.	Contact LC Packings

6.6 Spare Parts List

6.6.1 Major Items

Part Number	Description
	Standard Version (Stainless Steel Injection Valve)
160105	FAMOS Well Plate Microautosampler
161168	FAMOS Well Plate Microautosampler, with Cooling option 10 – 40 °C installed
161170	FAMOS Well Plate Microautosampler, with Feeder option installed
161171	FAMOS Well Plate Microautosampler, with Feeder and Cooling option 10 – 40 °C installed
	Inert Version (PAEK Injection Valve)
160614	FAMOS Well Plate Microautosampler, inert version
161169	FAMOS Well Plate Microautosampler, inert version, with Cooling option 10 – 40 °C installed
161172	FAMOS Well Plate Microautosampler, inert version, with Feeder option installed
161173	FAMOS Well Plate Microautosampler, inert version, with Feeder and Cooling option 10 – 40 °C installed
	Firmware
160108	Firmware, ERPOM and RAMs for FAMOS Well Plate

6.6.2 Needle Assembly

Part Number	Description
160152	Air/pre-puncturing needle, including seal
160153	Needle guide body
160120	Needle guide / Air nut for Fused Silica Needle
162019	Needle guide / Air nut for Conventional Needle
	Standard Version (Stainless Steel Injection Valve) - Micro Mode
160116	Fused silica injection (sample) needle, 100 µm I.D., pre-assembled (2.4 µL).
160117	Fused silica fraction/injection needle, 75 µm I.D., extra long version (10.3 µL)
	Standard Version (Stainless Steel Injection Valve) - Conventional Mode
160119	Standard (conventional) stainless steel sample needle, supplied with TEFZEL tubing (15µL)
	Inert Version (PAEK Injection Valve) - Micro Mode
162000	Fused silica injection (sample) needle, 100 µm I.D., pre-assembled (2.4 µL), for inert injection valve
	Inert Version (PAEK Injection Valve) - Conventional Mode
161474	Inert conventional needle for FAMOS, made of PEEK, for inert injection valve

6.6.3 Syringe and Buffer Tubing

Part Number	Description
160128	Syringe 25 µL
160129	Syringe 100 µL
160130	Syringe 250 µL
160131	Syringe 500 µL
160132	Syringe 1 mL
160137	Luer lock adaptor for FAMOS Well Plate Microautosampler
160155	Plunger replacement tip 25 µL (Set of 10)
160156	Plunger replacement tip 100 µL (Set of 10)
160157	Plunger replacement tip 250 µL (Set of 10)
160158	Plunger replacement kit 500 µL (Set of 10)
160159	Plunger replacement kit 1000 µL (Set of 10)
162020	Syringe waste tubing for FAMOS
162021	Syringe wash tubing for FAMOS
162022	Tubing SSV to syringe valve for FAMOS
160161	Flangeless ferrule 1/16" blue for "buffer" tubing connection to low dispensing valve
160160	Male nut 1/16" blue for "buffer" tubing connection to low pressure dispensing valve
	Standard Version (Stainless Steel Injection Valve)
160125	Buffer tubing 50 µL
160126	Buffer tubing 500 µL
160127	Buffer tubing 2000 µL
160143	Upgrade kit for FAMOS, for 100 µL syringe (b)
160142	Upgrade kit for FAMOS, for 250 µL syringe (b)
160141	Upgrade kit for FAMOS, for 500 µL syringe (b)
160140	Upgrade kit for FAMOS, for 1.0 mL syringe (b)
	Inert Version (PAEK Injection Valve)
161018	Buffer tubing 50 µL, for inert injection valve
161264	Buffer tubing 500 µL, for inert injection valve
162023	Buffer tubing 2000 µL, for inert injection valve
162024	Upgrade kit for FAMOS, for 100 µL syringe, inert version (b)
162025	Upgrade kit for FAMOS, for 250 µL syringe, inert version (b)
162026	Upgrade kit for FAMOS, for 500 µL syringe, inert version (b)
162027	Upgrade kit for FAMOS, for 1.0 mL syringe, inert version (b)

(b) includes Luer lock adapter (P/N 160137), syringe and buffer tubing

6.6.4 Injection Valve

Part Number	Description
	Standard Version (Stainless Steel Injection Valve)
160163	VALCO C2, 6-Port Micro Injection Valve ASSY
160149	Replacement rotor for VALCO C2, 6-Port Micro Injection Valve
160151	Stator VALCO C2, 6-Port Micro Injection Valve
160109	1 µL loop, FAMOS, PEEK shielded fused silica tubing
160110	5 µL loop, FAMOS, PEEK shielded fused silica tubing
160111	10 µL loop, FAMOS, PEEK shielded fused silica tubing
160112	20 µL loop, FAMOS, PEEK shielded fused silica tubing
160113	50 µL loop, FAMOS, stainless steel tubing
160114	100 µL loop, FAMOS, stainless steel tubing
161481	125 µL loop, FAMOS, stainless steel tubing
161482	250 µL loop, FAMOS, stainless steel tubing
161483	500 µL loop, FAMOS, stainless steel tubing
160115	Connecting tubing valve/micro column, 50 µl I.D., including low dispersion union
	Inert Version (PAEK Injection Valve)
161265	VALCO C2, 6-Port PAEK Micro Injection Valve ASSY
161003	Replacement rotor for VALCO C2, 6-Port Micro Injection Valve, inert version
161004	PAEK Stator for VALCO C2, 6-Port Micro Injection Valve, inert version
161015	1 µL loop for FAMOS inert, PEEK shielded fused silica tubing
161016	5 µL loop for FAMOS inert, PEEK shielded fused silica tubing
161017	10 µL loop for FAMOS inert, PEEK shielded fused silica tubing
162028	20 µL loop for FAMOS inert, PEEK shielded fused silica tubing
162035	50 µL loop for FAMOS inert, PEEK tubing
162029	100 µL loop for FAMOS inert, PEEK tubing
161263	125 µL loop for FAMOS inert, PEEK tubing
162030	250 µL loop for FAMOS inert, PEEK tubing
162031	500 µL loop for FAMOS inert, PEEK tubing
161499	Connecting tubing valve/micro column, 50 µl I.D., including low dispersion union, for inert injection valve

6.6.6 Vials and Accessories

Part Number	Description
162034	Wash vial for FAMOS
162032	Transport Vial 10 mL (20 x 45), 4 PCS
160134	Polypropylene vials for FAMOS, 250 µL, 1000 PCS
160133	Polypropylene vials for FAMOS, 250 µL, 100 PCS
161485	Polypropylene vials for FAMOS, 250 µL, 50 PCS
160136	Polypropylene caps for FAMOS, 1000 PCS
160135	Polypropylene caps for FAMOS, 100 PCS
161484	Polypropylene caps for FAMOS, 50 PCS
161493	Glass vials, 1.5 mL, 100 PCS
161494	Glass inserts, 200 µL, 100 PCS
160162	Wash solvent bottle 100 mL
162033	Wash solvent bottle 100 mL

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CHAPTER 7

Specifications

7.1 General

7.1.1 Analytical Performance

Capped and sealed vials	**Reproducibility**
full loop injections	RSD ≤ 0.4 %
partial loopfill injections	RSD ≤ 0.6 %, injection volumes ≥ 1 µL, with headspace pressure on the vial and 5 µL pre-flush
µL pick-up injections	RSD ≤ 1.5 %, injection volumes ≥ 1 µL, without headspace pressure on the vial

Open vials/plates [η = 1.0 cP]:	**Reproducibility**
full loop injections	RSD ≤ 0.3 %
partial loopfill injections	RSD ≤ 1.0%; injection volumes ≥ 1 µL, without headspace pressure on the well.
µL pick-up injections	RSD ≤1.5 %; injection volumes ≥ 1 µL, without headspace pressure on the vial.
Memory effect	< 0.01 % with programmable needle wash

7.1.2 Programming

Injection methods	Full loop injections Partial loopfill injections µL pick-up injections
Injection volume	0.01 µL - 1000 µL, full loop, depending on system settings 0.01 - 500 µL, with 0.1 µL increments for partial loopfill 0.1 µL - max. volume, with 0.01 µL increments for µL pick-up max. volume = ((loop volume - 3 x needle volume) /2)
Injections per vial	Max. 9 (volumes are programmable for each injection)
Analysis time	Max. 9 hrs. 59 min. 59 sec.
Needle wash	Programmable (between injections, wells or series)
Priority sample	Freely programmable
Series	Freely programmable, 24 series max

7.1.3 Inputs/Outputs

Digital Output	2 RS-232 2 EVENT: (Open Collector, Relay) START-, ERROR IN: Optocoupler START-, ERROR OUT: Open Collector
Analog Input	1 V (1V/100 nm)
Analog Output	2 ±0.1 V/ ±1 V / ±10 V (for recorder or integrator)

7.1.4 Physical

Dimensions (WxDxH)	280 mm (11.0 in) x 400 mm (15.7 in) x 440 mm (17.3 in).
Weight	23 kg (50.7 lb.).

7.1.5 Electrical

Power Requirements	115 VAC; + 15/-20 %; 50 Hz/60 Hz; 250 VA 230 VAC; + 15/-20 %; 50 Hz/60 Hz; 250 VA
Fuses	115 VAC; two 5.0 AT-fuses; (¼" x ¼", UL/CSA) 230 VAC; two 2.5 AT-fuses; (5 x 20 mm, IEC 127) All fuses UL-listed and CSA-certified

7.1.6 Communication

Outputs	Inject marker Well marker Labeled well marker Stop I/O 4 Auxiliary outputs 2 Programmable outputs Alarm output 4 Bit timebase
Inputs	Next injection input Next well input Freeze input Stop I/O 4 Programmable inputs
Serial Communication	via RS232C

7.1.7 Options

Sample cooling	Built-in Peltier cooling processing unit Programmable Range: 4° C - 40° C Cooling capacity: Ambient -20° C (measured on cooling plate, plate holder removed) Note: The temperature of the sample inside the sample vial may be slightly different than the temperature set via the command, due to a variety of effects (e.g. the heat transfer characteristics of the vial walls). If it is necessary to precisely set the temperature inside the vial, we recommend that you determine the temperature inside the vial at various temperature setpoints and set the cooling option so that the desired internal temperature is attained.
Column oven	THERMOS™ column oven combined with the FAMOS Well Plate Microautosampler. The injection valve and optional high pressure valve fit into the column oven.

7.1.8 Plate Dimensions

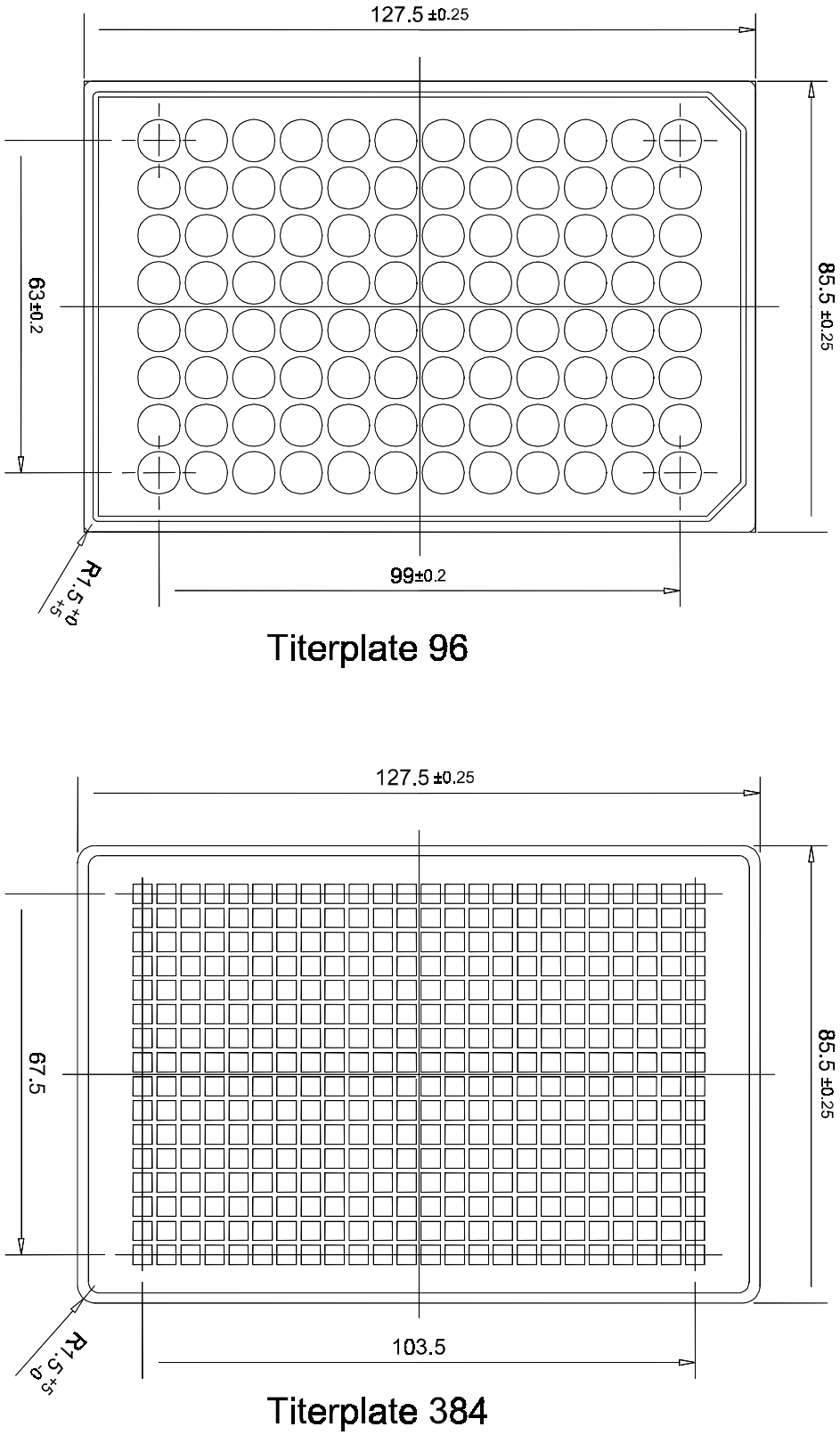


FIGURE 7-1: Titerplate Formats

APPENDIX A

Injection Principles

This appendix describes detailed information about the modes of injection used by the FAMOS™ Well Plate Microautosampler.

A.1 Full Loop Injections

A.1.1 Standard Mode

The switching sequence for a full loop injection is described in this section.

At the start of the sequence, the injector is in the INJECT position (FIGURE A-1) and the sample needle has entered the well after the air needle has pre-punctured the septum. Headspace pressure is applied through the outer air needle to ensure that no air or vapor bubbles are formed during sample aspiration.

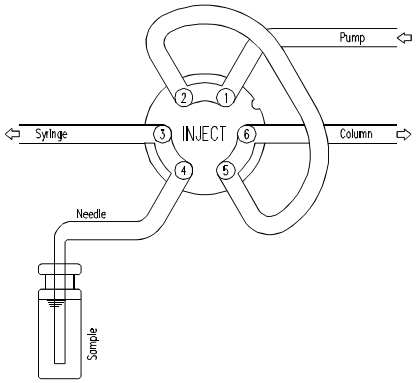


FIGURE A-1. Injector is in the INJECT Position

The syringe dispenser aspirates the programmed flush volume from the sample well to fill the sample line with sample and remove wash solvents (FIGURE A-2).

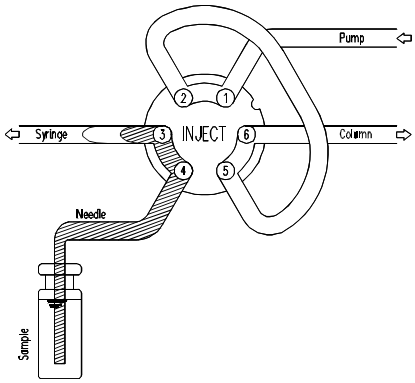


FIGURE A-2. Sample Line Filled with Sample

When the injection valve is switched into the LOAD position (FIGURE A-3), a "sharp" sample front is placed at the inlet of the sample loop.

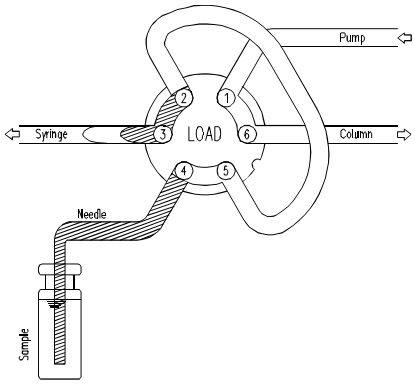


FIGURE A-3. Injection Valve Switched into LOAD Position

For full loop injections, the sample loop is quantitatively filled by transporting two or more times the loop volume through the loop (FIGURE A-4). The actual volume that is transported is dependent on the volume of the loop.

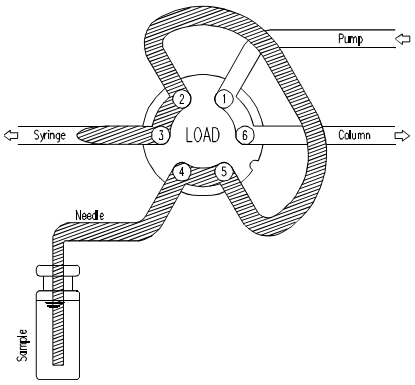


FIGURE A-4. Transporting Two (or more) Times the Loop Volume Through the Loop

The injection valve is then switched into the INJECT position (FIGURE A-5). The sample loop is now part of the HPLC mobile phase flow path so that the sample is transported to the column and the analysis time starts.

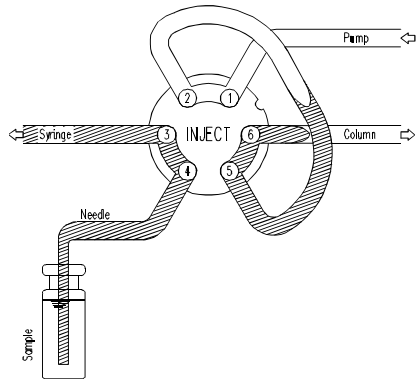


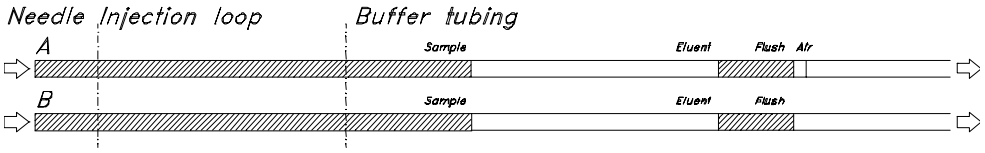
FIGURE A-5. The Injection Valve Switched into the INJECT Position

If one injection is to be done from each well or if a wash routine has to be performed after every injection, the needle withdraws from the well directly after the injection and, immediately washed (if programmed). After the analysis time is completed, a new sequence is started.

If more than one injection is done from the same well without a wash routine, the FAMOS Well Plate Microautosampler withdraws a flush volume after the analysis time to compensate for diffusion of mobile phase from the rotor groove into the first part of the sample line during the analysis time. The flush volume between injections is not programmable and is always 50% of the programmed flush volume. If the total amount of sample withdrawn with the next injection from the well will exceed the total volume of the buffer tubing, the buffer tubing is emptied into the wash position before the next injection. The next fill sequence will then start with a full flush volume.

A.1.2 Using an Air Segment

An air segment can be used to reduce the amount of flush volume. This air segment is at the front of the flush volume. It will not be injected and it will not influence the injection. Use of an air segment can be enabled in the System Menu (General Menu).



A = with air segment
B = without air segment

FIGURE A-6. Full Loop Injection with and without Air Segment

With the FAMOS Fused Silica needle, the flush volume must be at least 5.0 µL for injections without air segment (if a stainless steel needle is used, the flush volume should be at least 15.0 µL). If the samples are highly viscous, it may be necessary to program larger flush volumes and reduce the syringe speed for better performance.

A.2 Partial Loopfill Injections

A.2.1 Standard Mode

The switching sequence for a partial loopfill injection is schematically presented in this section. The first three steps are identical to those for Full Loop injections (see Section A.1).

For partial loopfill injections, the sample loop is filled by transporting the programmed injection volume into the sample loop (FIGURE A-7).

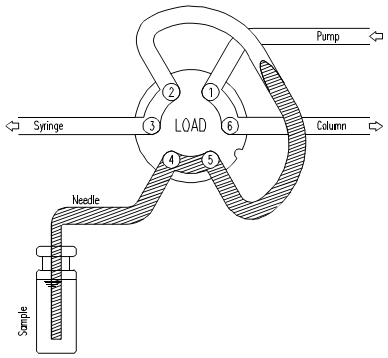


FIGURE A-7. Partially Filling the Injection Loop with the Programmed Injection Volume

The injection valve switches into the INJECT position (FIGURE A-8) and the sample loop is now part of the HPLC mobile phase flow path. At this point, the sample is transported to the column and the analysis time starts.

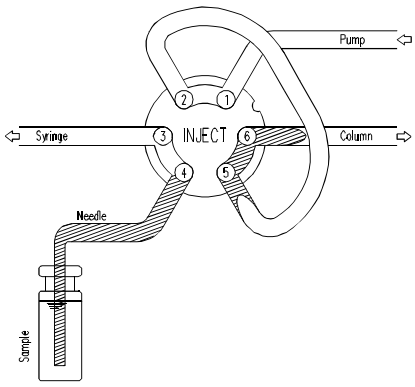
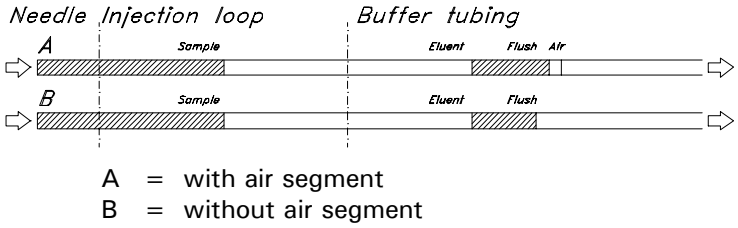


FIGURE A-8. The Injection Valve Switched into the INJECT Position

The next injection sequence will start with a flush of 50% of the programmed flush volume, if the next injection is from the same vial and no wash routine is programmed. Otherwise it will start with a flush of the programmed flush volume. If the aspiration of sample for the next injection will exceed the total volume of the sample buffer tubing, the buffer tubing is emptied before the next injection. The next injection will start with the programmed flush, as described for full loop injections.

A.2.2 Using an Air Segment

An air segment can be used to reduce the amount of flush volume (FIGURE A-9). This air segment is at the front of the flush volume and will not be injected. Use of an air segment can be enabled in the System Menu (General Menu).



A = with air segment
B = without air segment

FIGURE A-9. Partial Loop Injection with and without Air Segment

A.3 µL Pick-Up Injections

A.3.1 Standard Mode

The switching sequence for a µL pick-up injection is schematically presented in this section.

At the start of the sequence, the injection valve is in INJECT position (FIGURE A-10). The sample needle has entered the vial of transport liquid (normally mobile phase to avoid disturbance of the chromatogram with an additional peak of the transport solvent) after the air needle has pre-punctured the septum. The headspace pressure, applied through the outer air needle, ensures that no air or vapor bubbles are formed during wash solvent aspiration.

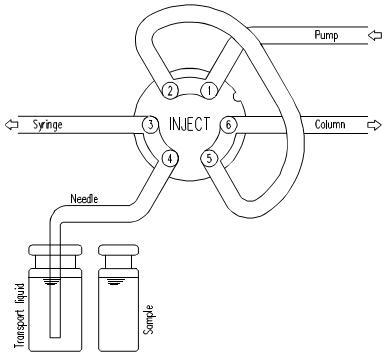


FIGURE A-10. Injection Valve in Inject Position, Sample Needle in the Transport Vial

Note: When performing µL pick-up injections, use the mobile phase as the transport liquid to avoid a disturbance of the chromatogram due to an additional peak of the transport solvent).

For the first injection after a wash or after emptying of the buffer tubing, the syringe dispenser aspirates transport liquid from the transport vial to fill the sample line with transport liquid and remove wash solvent (FIGURE A-11).

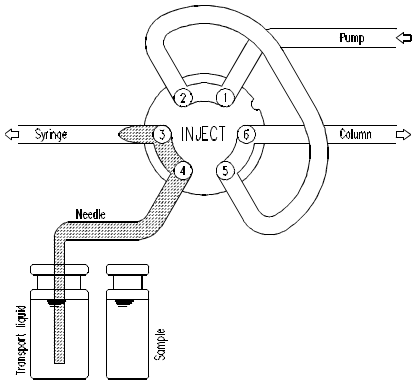


FIGURE A-11. The Syringe Dispenser Aspirating Transport Liquid to Fill Sample Line

The needle moves from the transport vial to the sample well (FIGURE A-12). The injection valve is switched to the LOAD position.

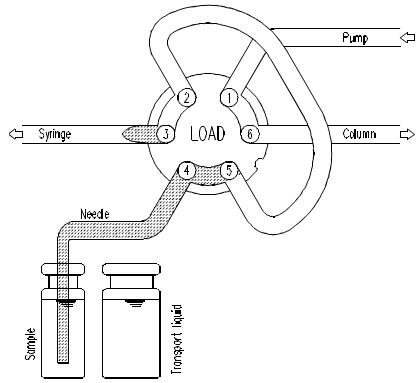


FIGURE A-12. The Injection Valve is Switched into LOAD Position

The programmed injection volume is aspirated from the sample well (FIGURE A-13).

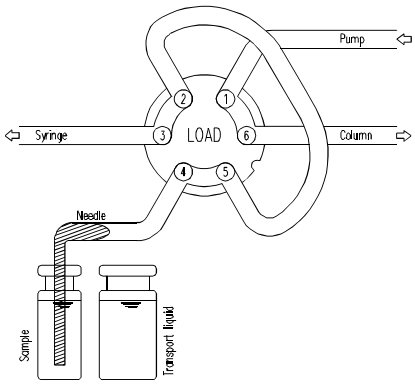


FIGURE A-13. Aspirating the Programmed Injection Volume

The sample needle moves back to the transport vial (FIGURE A-14). The sample is quantitatively transported into the loop, with transport liquid (mobile phase) from the transport vial.

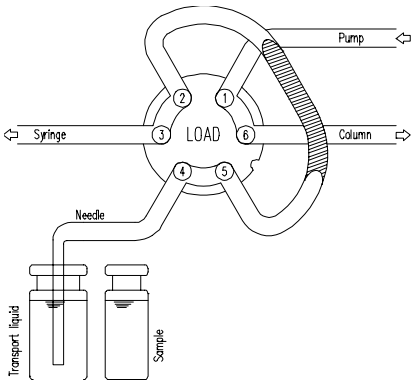


FIGURE A-14. Transporting Sample into the Injection Loop (Aspirating the Programmed Injection Volume)

The injection valve is switched to INJECT (FIGURE A-15). The sample loop is now part of the HPLC mobile phase flow path and sample is transported to the column. The analysis time is started.

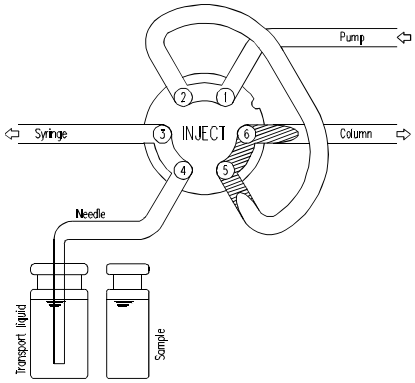


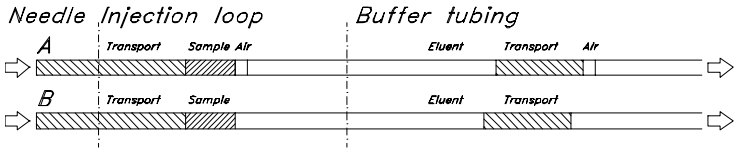
FIGURE A-15. The Injection Valve in the INJECT Position (Aspirating the Programmed Injection Volume)

The next sequence will skip the first withdrawal of transport solvent, unless a wash routine is performed or the FAMOS Well Plate Microautosampler has emptied the buffer tubing to waste. In those cases the sequence is completely repeated.

A.3.2 Using an Air Segment

If an air segment has been programmed, it appears at the front of the first plug of transport liquid and at the front of every sample plug. Use of an air segment can be enabled in the System Menu (General Menu).

Note: The air segment at the front of the sample plug is injected into the HPLC system.



A = with air segment
B = without air segment

FIGURE A-16. µL Pick-up Injections with and without Air Segment

A.4 Low Dispersion Injection

In addition to the standard injection methods, the FAMOS Well Plate Microautosampler allows the analyst to control the injection profile via the "Low Dispersion" routine in the partial loopfill and the full loopfill injection modes.

The injection principle is used to improve the injection profile (and therefore improve the reproducibility) by switching the injection valve back into load position after a calculated time and to cut off the tailing part of the sample plug.

Two parameters are used to control the Low Dispersion mode:

Flow Rate: This value determines the flow rate at which the sample is flushed out of the injection loop. This value corresponds to the flow rate of the LC system. The valid range is 00.00 to 99.99 µL/min

☞ **Note: If the flow rate is set to 00.00 µL/min, a standard injection is performed.**

Low Dispersion Factor (L.D. factor): This value determines the amount of sample volume which is cut off by the back switching injection valve. A value of 1.0 corresponds to the volume of the sample plug. If the value is greater than (less than) 1.0, the injection valve will switch back after the volume is greater than (less than) the sample volume which has been transferred through the sample loop. The valid range is 0.7 to 2.0. As an example, if the value is 1.2, the valve will switch back after 120 % of the value of the sample plug has been transferred. The injection profile for various values is shown in FIGURE A-17.

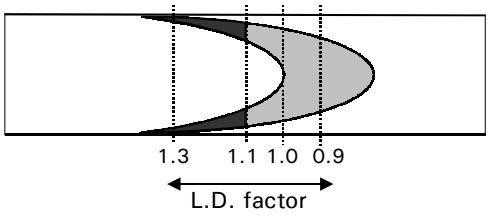


FIGURE A-17. Low Dispersion Injection Profiles

The time the injection valve is switched back into the load position is calculated by the equation A-1.

$$S_t = (f)\ (V_1)\ (60)/F \qquad \text{A-1}$$

where: S_t = Switching Time (sec), f = L.D. Factor,
V_1 = Injection Volume (μL), F = Flow Rate (μL/min)

As an example: If V_{inj} = 1 µL, the flow rate is 5 µL and the L.D. factor is 1.1, the valve switching time S_t will be 13.2 s.

☞ **Note: Under these conditions the loop will be flushed with the wash solvent as well, which may result in injection problems (e.g. lack of proper focussing). It is recommended that the mobile phase (typically mobile phase A) is used as the wash solvent.**

☞ **Note: The injection time must be at least 10 s. If the calculated injection time is less than this limit, it will be redefined to 10 s.**

A.5 Comparing the Various Sample Injection Modes

The characteristics of each injection mode is described below:

- **Full loop injection** - provides maximum reproducibility (RSD < 0.4%), but not maximum accuracy, since loop volume is specified with an accuracy of ± 10%. Minimum sample loss = 12.4 µL (2 x loop overfill + flush volume for needle) for the standard 5 µL loop.

- **Partial loopfill** - provides maximum accuracy (depends on syringe accuracy) and reproducibility better than 0.6% RSD for injection volumes > 1 µL. Minimum sample loss (Flush volume) = 5.0 µL. 5.0 µL is the recommended minimum flush volume; smaller flush volumes can be programmed, but will result in decreased performance. For maximum reproducibility and accuracy a 25 µL syringe should be used and a 1 µL sample loop should be used to avoid loss of accuracy due to expansion of the loop content when switching from inject to load position prior to sample loading. When working with high pressure (200 bar), this loss may be up to 0.025 µL for a 5 µL loop.

Note: The minimum sample loss in partial loopfill mode is 5 µL (recommended minimum flush volume) for the first injection and an additional 2.5 µL (always half the programmed flush volume) for additional injections from the same well. If a wash between injections has been programmed, sample loss is 5 µL for every injection. For zero sample loss injections, use the µL-pick injection mode.

- **µL Pick-up** - means zero sample loss, maximum accuracy (same as partial loopfill), but slightly diminished reproducibility: RSD better than 1.5% for injection volumes ≥ 1 µL (sample volume must be larger then 10 µL). For optimum accuracy and reproducibility, a 25 µL syringe and a 10 µL sample loop should be used. The sample plug is transported into the loop, preceded by a plug of transport liquid that has a volume of 2.5 times the programmed needle tubing volume.

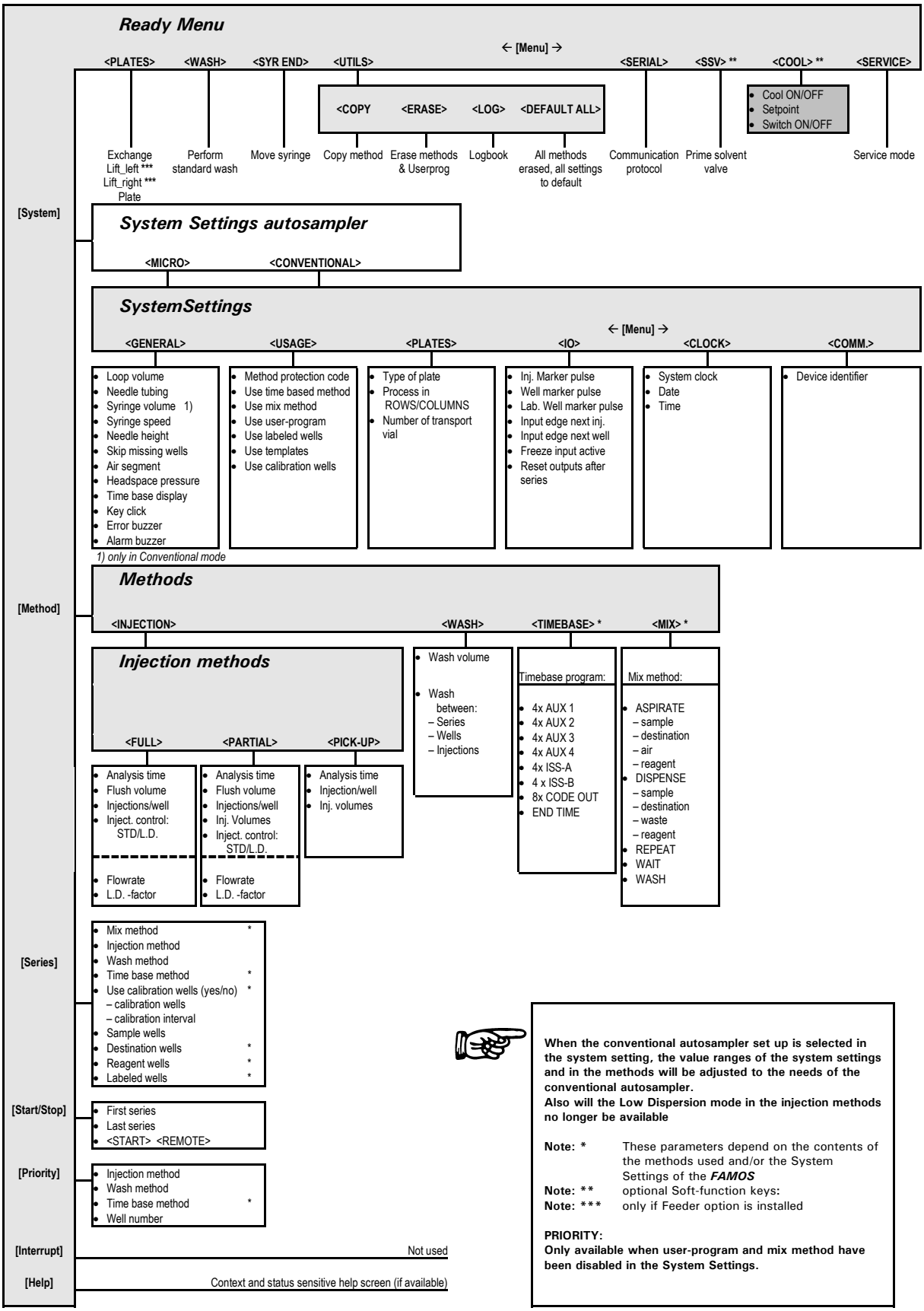
APPENDIX B

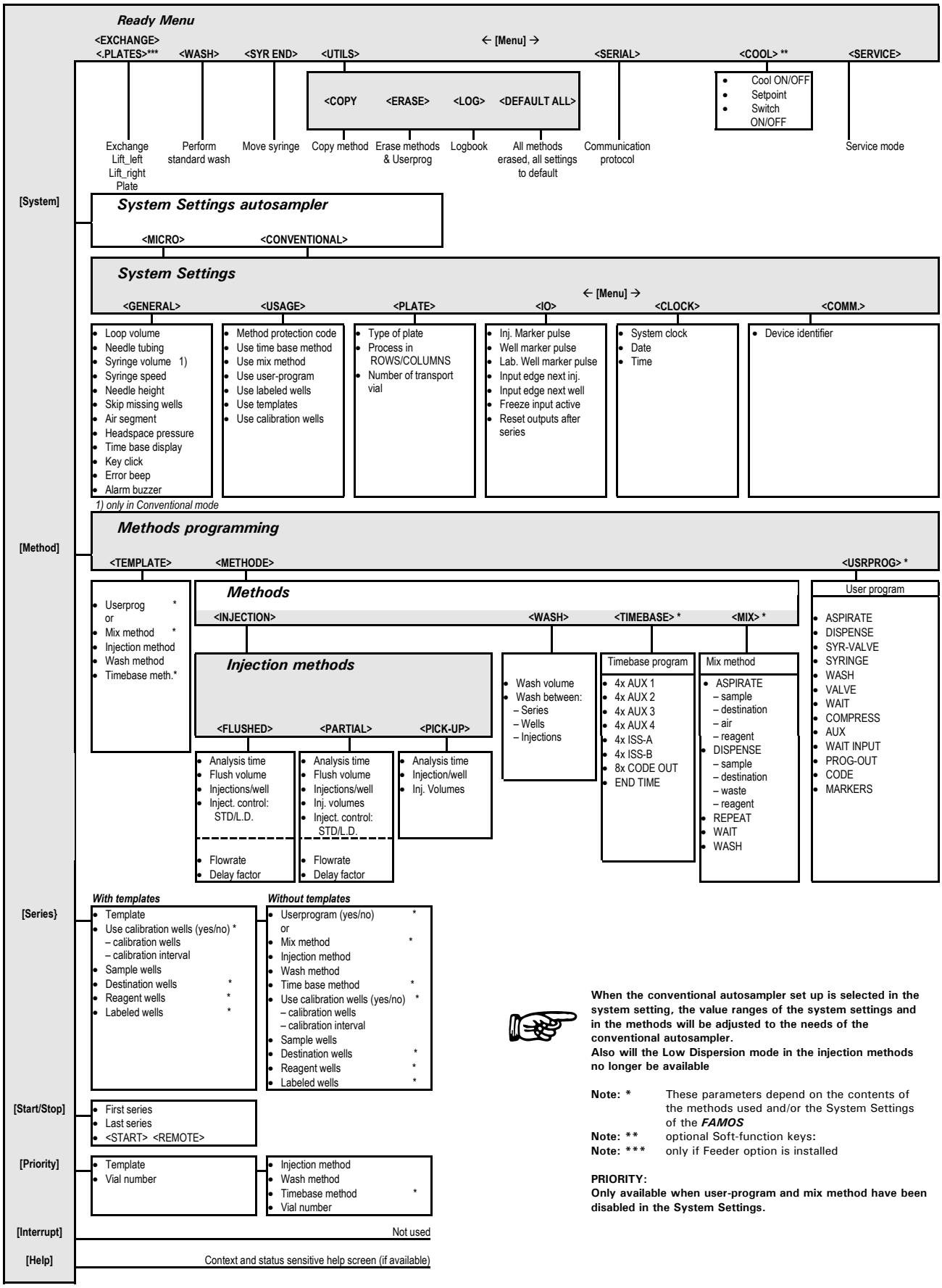
Programming Charts

B.1 Overview of the Operating Program

The details of the operating program for the FAMOS Well Plate Microautosampler is described in detail in Chapter 3, which includes a discussion of each parameter, the allowable values and its role in the overall operation of the autosampler.

The tables in this appendix are provided to allow the user to get an overview of the various commands in each menu and how the various commands are accessed.


Ready Menu
← [Menu] →
<PLATES>
<WASH>
<SYR END>
<UTILS>
<SERIAL>
<SSV> **
<COOL> **
<SERVICE>
<COPY
<ERASE>
<LOG>
<DEFAULT ALL>
Cool ON/OFF
Setpoint
Switch ON/OFF
Exchange Lift_left *** Lift_right *** Plate
Perform standard wash
Move syringe
Copy method
Erase methods & Userprog
Logbook
All methods erased, all settings to default
Communication protocol
Prime solvent valve
Service mode
[System]
System Settings autosampler
<MICRO>
<CONVENTIONAL>
SystemSettings
← [Menu] →
<GENERAL>
<USAGE>
<PLATES>
<IO>
<CLOCK>
<COMM.>
Loop volume
Needle tubing
Syringe volume 1)
Syringe speed
Needle height
Skip missing wells
Air segment
Headspace pressure
Time base display
Key click
Error buzzer
Alarm buzzer
Method protection code
Use time based method
Use mix method
Use user-program
Use labeled wells
Use templates
Use calibration wells
Type of plate
Process in ROWS/COLUMNS
Number of transport vial
Inj. Marker pulse
Well marker pulse
Lab. Well marker pulse
Input edge next inj.
Input edge next well
Freeze input active
Reset outputs after series
System clock
Date
Time
Device identifier
1) only in Conventional mode
[Method]
Methods
<INJECTION>
<WASH>
<TIMEBASE> *
<MIX> *
Injection methods
<FULL>
<PARTIAL>
<PICK-UP>
Analysis time
Flush volume
Injections/well
Inject. control: STD/L.D.
Flowrate
L.D. -factor
Analysis time
Flush volume
Injections/well
Inj. Volumes
Inject. control: STD/L.D.
Flowrate
L.D. -factor
Analysis time
Injection/well
Inj. volumes
Wash volume
Wash between:
– Series
– Wells
– Injections
Timebase program:
4x AUX 1
4x AUX 2
4x AUX 3
4x AUX 4
4x ISS-A
4 x ISS-B
8x CODE OUT
END TIME
Mix method:
ASPIRATE
– sample
– destination
– air
– reagent
DISPENSE
– sample
– destination
– waste
– reagent
REPEAT
WAIT
WASH
[Series]
Mix method *
Injection method
Wash method
Time base method *
Use calibration wells (yes/no) *
– calibration wells
– calibration interval
Sample wells
Destination wells *
Reagent wells *
Labeled wells *
[Start/Stop]
First series
Last series
<START> <REMOTE>
[Priority]
Injection method
Wash method
Time base method *
Well number
[Interrupt]
Not used
[Help]
Context and status sensitive help screen (if available)
When the conventional autosampler set up is selected in the system setting, the value ranges of the system settings and in the methods will be adjusted to the needs of the conventional autosampler.
Also will the Low Dispersion mode in the injection methods no longer be available
Note: * These parameters depend on the contents of the methods used and/or the System Settings of the FAMOS
Note: ** optional Soft-function keys:
Note: *** only if Feeder option is installed
PRIORITY:
Only available when user-program and mix method have been disabled in the System Settings.



When the conventional autosampler set up is selected in the system setting, the value ranges of the system settings and in the methods will be adjusted to the needs of the conventional autosampler.
Also will the Low Dispersion mode in the injection methods no longer be available

Note: * These parameters depend on the contents of the methods used and/or the System Settings of the ***FAMOS***
Note: ** optional Soft-function keys:
Note: *** only if Feeder option is installed

PRIORITY:
Only available when user-program and mix method have been disabled in the System Settings.

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APPENDIX C

Additional Programming Examples

C.1 Overview

The commands described in Chapter 3 can be used by the analyst to generate a broad range of programs for the operation of the FAMOS™ Well Plate Microautosampler. These commands allow the user to configure the unit to meet the specific needs of the laboratory. A series of blank forms are provided to assist the analyst in programming (Appendix F).

Chapter 4 describes a number of programs that the user can prepare for activities that are commonly performed with the microautosampler. In this appendix, we describe a few programs that are important, but used less frequently. In addition to the direct use of these programs, they can be used to provide an understanding of the logical processes that are used in generating a program.

The following programs are included in this appendix.

- A 1.0 µL partial loopfill (Section C.2)
- A 3 x 1.0 µL injection with µL pick-up partial loopfill injection (Section C.3)
- A 1:10 dilution followed by a 1.0 µL partial loopfill injection (Section C.4)
- Defining a Template and adding a protection code (Section C.5)
- 1 µL injections out of 25 wells using a 384 well plate (Section C.6)
- Column Switching (Section C.7)

It should be noted that the examples described below could be used with systems that include *UltiMate*™ with the *UltiChrom*™ software program as well as for stand-alone systems.

C.2 A 1.0 µL Partial Loopfill

This example describes an application in which a 1.0 µL injection is made from a single vial in position 1. In this example, we assume that a 5 µL loop, a 2.4 µm needle and a 25 µL syringe have been installed.

a) Place a sample in position A1.

b) Program the System Parameters by entering the parameters indicated in TABLE C-1.

TABLE C-1. Programming System Parameters

Press keys	Description
System	to enter the System Menu
<MICRO> **E**	to select the micro mode
<GENERAL> **E**	to enter the General Menu
[05.0] **E**	to define the volume of the installed loop
[02.4] **E**	to define the volume of the needle tubing
<LOW> **E**	to set syringe speed to low
[02] **E**	to set sample needle height to 2 mm
<NO> **E**	to enable use of air segment
<YES> **E**	to switch on headspace pressure
Escape	to return to the System Menu
<PLATES> **E**	to enter the Plates Menu
<96-LOW>	to define the type of plate to be used
<IN ROWS>	to define the processing order of wells
Escape Escape	to return to the Ready Menu

For this example, all other settings used will be the default value. In the tables presented in this appendix the name of the soft key to be selected is indicated in angle brackets (e.g. <PLATES>), and the value which is indicated in square brackets (e.g. [05.0]). The **bold** letters indicates that you have to press a function key (e.g. **E** which represents Enter).

c) Program the method by entering the parameters indicated in TABLE C-2.

TABLE C-2. Programming Method Parameters

Press keys	Description
Methods	to enter the Methods Menu
<INJECTION> [01] **E**	program injection method number 1
<PARTIAL> **E**	to select partial loopfill injection method
[100] **E**	to define an analysis time of 1 minute
[05.0] **E**	to define a flush volume of 5.0 µL
[1] **E**	to define the number of injections per well
[1.00] **E**	to set the injection volume at 1.00 µL
Escape Escape	to return to the Ready Menu

d) Program the series by entering the parameters indicated in TABLE C-3.

TABLE C-3. Programming Series Parameters

Press keys	Description
Series	to enter the Series Menu
[01] **E**	to define the Series number
[01] **E**	to define the injection method number
CL E	to enter <NONE> for wash method
<ROW A> [01] **E**	to define location of the first sample well
<ROW A> [01] **E**	to define location of the last sample well
Escape	to return to the Ready Menu

e) Run the series by entering the parameters indicated in TABLE C-4 and pressing <START>.

TABLE C-4. Running the Series

Press keys	Description
Start/Stop	to start the FAMOS Well Plate Microautosampler
[01] **E**	to start at series number 1
[01] **E**	to stop after execution of series number 1
<START>	to start the analytical run

The FAMOS Well Plate Microautosampler will now locate well A01 and perform a 1.0 μL partial loopfill injection. The display of the FAMOS Well Plate Microautosampler will indicate the status (Checking tray, Flushing, Loopfill, Running, Rinse buffer, Running). The display also indicates the number of the defined series (01), the method number (01) and the well on which the analysis is performed (A01).

At the end of the defined analysis time, the Ready Menu will be displayed again to indicate that the microautosampler is ready for the next analytical run.

C.3 A 3 x 1.0 µL Injection with µL Pick-Up and Wash between Injections

This example describes an application where three 1.0 µL injections are made, with a wash between each injection.

a) Place a vial with transport solvent (mobile phase) in transport vial position 1 (left) and place the sample in position A1. Make sure the transport vial is correctly filled before starting a new series.

b) Program the System Parameters by entering the parameters indicated in TABLE C-5.

TABLE C-5. Programming System Parameters

Press keys	Description
System	to enter the System Menu
<MICRO> **E**	to select the micro mode
<GENERAL> **E**	to enter the General Menu
E until 'Air segment' appears	to go to the Air segment field
<NO> **E**	to switch off air segment
Escape	to return to the System Menu
<PLATES> **E**	to enter the Plates Menu
E E	to go to transport vials field
[01] **E**	to define position of the first transport vial
[01]	to define position of the last transport vial
Escape Escape	to return to the Ready Menu

e) Program the method by entering the parameters indicated in TABLE C-6.

TABLE C-6. Programming Method Parameters

Press keys	Description
Methods	to enter the Methods Menu
<INJECTION> [02] **E**	to define method number 02
<PICK-UP> **E**	to select the injection mode for this method
[100] **E**	to define the analysis time
[3] **E**	to define the number of injections per well
[100] **E**	to define volume of 1.0 µL for 1st injection
[100] **E**	to define volume of 1.0 µL for 2nd injection
[100] **E**	to define volume of 1.0 µL for 3rd injection
Escape	to return to the Methods Menu
<WASH>	to enter the Wash Menu
[01] **E**	to define wash method number 01
<INJECTION> **E**	to select wash between injections
[50]	to define the wash volume
Escape Escape	to return to the Ready Menu

Note: Make certain to indicate that a 10 µl loop is used in the system settings when µL pick-up injections are selected (Section 3.8.2).

f) Program the series by entering the parameters indicated in TABLE C-7.

TABLE C-7. Programming Series Parameters

Press keys	Description
Series	to enter the Series Menu
[01] **E**	to define the series number
[02] **E**	to define the injection method for this series
[01] **E**	to define the wash method for this series
<ROW A> [01] **E**	to define the location of the first sample well
<ROW A> [01]	to define the location of the last sample well
Escape	to return to Ready Menu

g) Run the series by entering the parameters indicated in TABLE C-8 and pressing <START>.

TABLE C-8. Running the Series

Press keys	Description
Start/Stop	to start the FAMOS Well Plate Microautosampler
[01] **E**	to start at series 01
[01] **E**	to stop after series 01
<START>	to start execution of the series.

At the end of the defined analysis time the Ready Menu will appear again to indicate that the FAMOS Well Plate Microautosampler is ready for the following next run.

C.4 A 1:10 Dilution followed by a 1.0 µL Partial Loopfill Injection

This example describes how to let the FAMOS Well Plate Microautosampler transfer 9 µL from Reagent A to the destination vial, add 1 µL of sample, followed by subsequently inject 1.0 µL (3 times). In addition, a 96 deep well plate is used and some wait steps are included to increase the performance.

a) Place a sample vial in position A 01, place an empty sample vial in position B 01 as destination vial. Place a filled reagent vial in position 1. Make sure the reagent vial is filled correctly before starting a new series.

b) Program the System Parameters by entering the parameters indicated in TABLE C-9.

TABLE C-9. Programming System Parameters

Press keys	Description
System	to enter the System Menu
<CONVENTIONAL> **E**	to select the conventional mode
<USAGE> **E**	to enter the Usage Menu
E E	to go to the Mix field
<ENABLED>	to enable use of mix methods
Escape	to return to the System Menu
<PLATES> **E**	to enter the Plates Menu
<96-HIGH>	to select 96 deep well plate
<COLUMNS>	to select processing in columns (A1, B1, etc)
CL	to disable use of transport vials
Escape Escape	to return to the Ready Menu

Note: As soon as a change has been entered in the System settings, the message "ALL SERIES DEFAULT" appears. The user will have to redefine series because the settings have been changed.

c) Program the method by entering the parameters indicated in TABLE C-10.

TABLE C-10. Programming Methods Parameters

Press keys	Description
Methods	to enter the Methods Menu
<INJECTION> [03] **E**	to enter the Injection Menu
<PARTIAL> **E**	to select partial loopfill injection mode
[100] **E**	to define the analysis time
[50] **E**	to define the flush volume
[3] **E**	to define the number of injections per well
[100] **E**	to enter the injection volume for 1st injection
[100] **E**	to enter the injection volume for 2nd injection
[100] **E**	to enter the injection volume for 3rd injection
Escape	to return to the Methods Menu

d) Program the mix method by entering the parameters indicated in TABLE C-11.

TABLE C-11. Programming Mix Methods Parameters

Press keys	Description
<MIX> [1] **E**	to enter the Mix Menu and define Mix method no. 1
<INSERT> <ASPIRATE> [100] **E** **Menu** <REAG-A> **E**	to define mix method step number 1 to aspirate 10.0 µL from reagent vial A
<INSERT> <DISPENSE> [90] <DESTINATION>	to define mix method step number 2 to dispense 9.0 µL to destination well
<INSERT> <WAIT> [02] **E**	to define mix method step number 3 to wait 2s
<INSERT> <DISPENSE> [10] <WASTE>	to define mix method step number 4 to dispense 1.0 µL to waste
<INSERT> <WAIT> [02] **E**	to define mix method step number 5 to wait 2s
<INSERT> <ASPIRATE> [20] **E** <AIR>	to define mix method step number 6 to aspirate 2.0 µL air
<INSERT> <WAIT> [02] **E**	to define mix method step number 7 to wait 2s
<INSERT> <ASPIRATE> [20] **E** <SAMPLE>	to define mix method step number 8 to aspirate 2.0 µL sample
<INSERT> <WAIT> [02] **E**	to define mix method step number 9 to wait 2s
<INSERT> <DISPENSE> [10] **E** <DESTINATION>	to define mix method step number 10 to dispense 1.0 µL to the destination well
<INSERT> <WAIT> [02] **E**	to define mix method step number 11 to wait 2s
<INSERT> <DISPENSE> [30] **E** <WASTE>	to define mix method step number 12 to dispense 3.0 µL to waste
<INSERT> <MENU> WASH [300] **E**	to define mix method step number 13 to perform a wash with 300 µL
<INSERT> <ASPIRATE> [20] **E** <AIR>	to define mix method step number 14 to aspirate 2.0 µL air
<INSERT> <ASPIRATE> [50] <DESTINATION>	to define mix method step number 15 to aspirate 5.0 µL from the destination well (note: step will improve
<INSERT> <DISPENSE> [50] <DESTINATION>	to define mix method step number 16 to dispense 5.0 µL to the destination well (step 15 and 16 are used to mix the destination)
<INSERT> <REPEAT> [2] ◂ [2] **E**	to define mix method step number 17 to repeat last 2 steps 2 times
<INSERT> <DISPENSE> [20] **E** <WASTE>	to define mix method step number 18 to dispense 2.0 µL to waste
<INSERT> <MENU> <WASH> [300] **E**	to define mix method step number 19 to perform a wash with 300 µL

e) Program the series by entering the parameters indicated in TABLE C-12.

TABLE C-12. Programming Series Parameters

Press keys	Description
Series	to enter the Series Menu
[01] **E**	to define series number 1
[01] **E**	to select Mix method number 1 for this series
[03] **E**	to select Injection method number 3
CL E	to select <NONE> for wash method
<ROW A> [1] **E**	to define location of first sample well
<ROW A> [1] **E**	to define location of last sample well
<ROW B> [1] **E**	to define location of first destination well
[1] **E**	to define position 1 for the Reagent A vial
Escape	to return to the Ready Menu

f) Run the series by entering the parameters indicated in TABLE C-13 and pressing <START>.

TABLE C-13. Programming System Parameters

Press keys	Description
Start/Stop	to start the FAMOS Well Plate
[01] **E**	to start at series number 1
[01] **E**	to stop after series number 1
<START>	to start processing of sample

The FAMOS Well Plate will now start searching for the Reagent vial and transport 90 µL to the destination well once, then 1 µL of sample will be added and after mixing 3 times a 1 µL injection will be performed.

C.5 Defining a Template and Adding a Protection Code

This example describes how to incorporate an injection method (02) and a wash method (01) which were defined in Section C.3 into a template. A protection code will be added.

After use of templates has been enabled, the message "ALL SERIES DEFAULT" appears. The user will have to redefine the series because the settings have been changed.

a) To select the methods to be incorporated in the template, enter the parameters described in TABLE C-14:

TABLE C-14. Programming the Method

Press keys	Description
Methods	to enter the Methods Menu
[123456] **E**	to enter the methods protection code
<TEMPLATE>	to enter the Template Menu
[01] **E**	to define the number for the template
[02] **E**	to define the injection method for this template
[01]	to define the wash method for this template
Escape Escape	to return to the Ready Menu

b) Program the series by entering the parameters indicated in TABLE C-15.

TABLE C-15. Programming Series Parameters

Press keys	Description
Series	to enter the Series Menu
[01] **E**	to define the Series number
[01] **E**	to define the Template method number
<ROW A> [01] **E**	to define the first sample well
<ROW B> [01] **E**	to define the last sample well
Escape	to return to the Ready Menu

c) Run the series by entering the parameters indicated in TABLE C-16 and pressing <START>.

TABLE C-16. Running the Series

Press keys	Description
Start/Stop	to start the Microautosampler
[01] **E**	to start analysis at series 01
[01] **E**	to stop after analysis of series 01
<START>	to start the analytical run

The FAMOS Well Plate Microautosampler now performs the same actions as in Example 2, except that analysis is performed on two wells: A 01 and B 01.

Note: Select <DEFAULT ALL> in the Ready Menu (Utilities Menu) to erase all *series* and *methods* defined in these examples and to default all settings.

C.6 1µL injections out of 25 wells of a 384 well plate

This example describes the injection of 1 µL from out of 25 wells from a 384 well plate.

a) Place the samples in well positions B5-B14, G14-G22 and M3-M8.

b) Enter the systems settings command from the <GENERAL> menu and select the 384 well plate.

c) Program the injection method as shown in TABLE C-17.

TABLE C-17. **Programming the Injection Method**

Press keys	Description
Methods	to enter the Methods Menu
<INJECTION> [01] **E**	program injection method number 1
<PARTIAL> **E**	to select partial loopfill injection method
[25.00] **E**	to define an analysis time of 25 minutes
[05.0] **E**	to define a flush volume of 5.0 µL
[1] **E**	to define the number of injections/vial
[1.00] **E**	to set the injection volume to 1.00 µL
<STD> **E**	to select the standard injection control
Escape Escape	to return to the Ready Menu

d) Program the three series as shown in TABLE C-18.

TABLE C-18. Programming Series Parameters

Press keys	Description
Series	to enter the Series Menu
[01] **E**	to define the Series number
[01] **E**	to select the injection method number
CL E	to enter <NONE> for wash method
<ROW B> [05] **E**	to define location B5 as first sample vial
<ROW B> [14] **E**	to define location B14 as last sample vial
Series	to enter the Series Menu
[02] **E**	to define the next Series number
[02] **E**	to select the next injection method number
CL E	to enter <NONE> for wash method
<ROW G> [14] **E**	to define location G14 as first sample vial
<ROW G> [22] **E**	to define location G22 as last sample vial
Series	to enter the Series Menu
[03] **E**	to define the next Series number
[03] **E**	to select the next injection method number
CL E	to enter <NONE> for wash method
<ROW M> [03] **E**	to define location M3 as first sample vial
<ROW M> [08] **E**	to define location M8 as last sample vial
Escape	to return to the Ready Menu

e) Run the series by entering the parameters indicated in TABLE C-19 and pressing <START>.

TABLE C-19. Running the Series

Press keys	Description
Start/Stop	to start the FAMOS Well Plate Microautosampler
[01] **E**	to start at series 01
[03] **E**	to stop after series 01
<START>	to start execution of the series.

At the end of the defined analysis time, the Ready Menu will reappear to indicate that the FAMOS Well Plate Microautosampler is ready.

C.7 Column Switching

The FAMOS Well Plate Microautosampler is also available with an additional ISS valve (Integrated Stream Switch option) which can be used for column switching experiments. In this section we provide an example of the use of a pre-column prior to the capillary HPLC analysis.

The following equipment is needed to perform this column switching example:

- A FAMOS Well Plate Microautosampler with:
  - the ISS-A valve (ISS in position A)
  - a 1000 µL syringe (cat. no. FMS-SY-1000)
  - a 2000 µL buffer tubing (cat. no. FMS-BT-2000)
  - a 250 µL injection loop
  - a standard injection needle assembly (cat. no. FMS-NDL-CNV) installed

- An additional HPLC pump for loading the sample at a flow rate of 0.5 mL/min.

- A µ-precolumn holder (cat. no. HD-05) and precolumns, e.g. 500 µm I.D. x 5 mm, 5 µm C18 (cat. no. MCA-50-C18).

To prepare the system:

- Replace the injection needle assembly (Section 6.3.3).

- Replace the 25 µL syringe for the 1000 µL syringe (Section 6.3.1) and replace the buffer tubing.

- Connect the contact closure input of the data system to the AUX 1 contact of the autosampler (P5 connector) in order to start the data acquisition at the moment the pre-column is switched on-line.

- Set the system up according to FIGURE C-1.

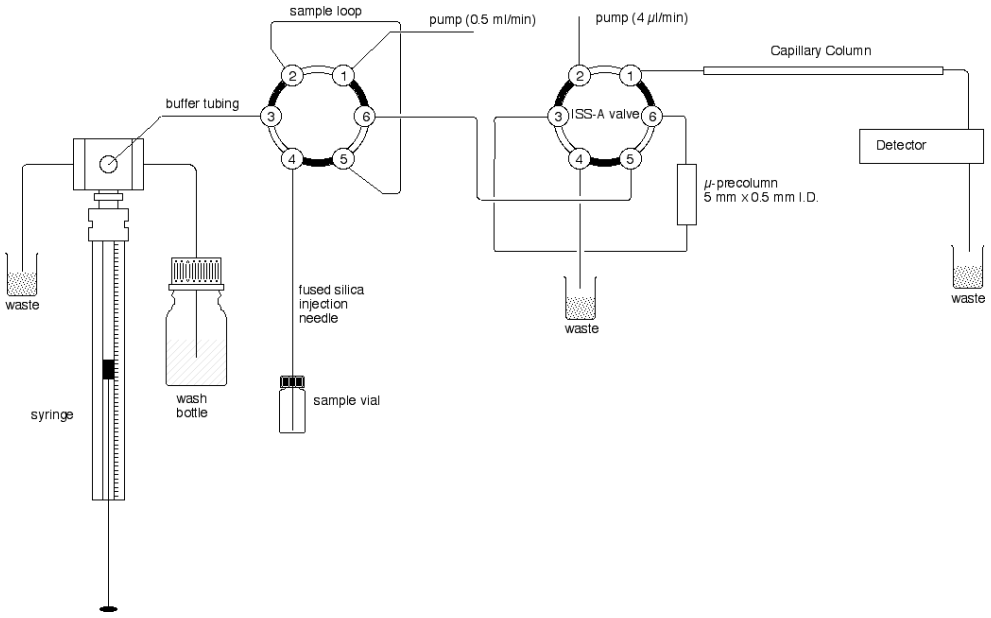

FIGURE C-1. Column Switching Setup

- Set the FAMOS Well Plate Microautosampler in <CONVENTIONAL> mode and adjust the general system parameters according to the modified setup (e.g. syringe, loop volume (Section 4.2).

- Enable the use of "time based methods" to allow programming of the AUX 1 contact closure (Section 3.8.4 and 3.8.12).

- Program "injection method 01" with the following parameters:
  "full loop" injection
  "flush volume": 30 µL
  "number of injections per vial": 1

- Program "Time Base 01" with the parameters shown in TABLE C-20.

TABLE C-20. Programming Time Base

Parameter	Time	Comment
ISS-A 1-6	00:45	switches pre-column on-line after sampler loading
ISS-A 1-2	14:50	switches the ISS-A valve back and the pre-column off-line again for the next sample
AUX-1	00:45	starts data acquisition of the data system
AUX-1	00:46	resets the contact closure output

- Program "Series 01" using "Injection Method 01" and "Time Base Method 01"

- Start the FAMOS Well Plate Microautosampler.

Note: The above example shows how to perform column switching with the FAMOS Well Plate Microautosampler equipped with one ISS valve. For advanced column switching applications (e.g. on-line digestion of proteins, on-line removal of non-ionic detergents from protein/peptide samples) LC Packings offers the SWITCHOS I (equipped with two valves) and the Switchos II (equipped additionally with a Loading Pump and Solvent Selection Valve) Advanced Micro Column Switching Units. Please contact LC Packings for additional information.

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APPENDIX D

The Sample Injector

D.1 Overview

The Cheminert® Model C2 Injection Valve is a 6-port external loop injector manufactured by Valco Instruments, Co. Inc. It is designed to offer optimal results with any of the injection principles used in the FAMOS™ Well Plate Microautosampler, including the partial filling and the µL Pick-Up methods (in which the injection volume is determined by the syringe) and full loop injection (in which the volume is determined by the size of the loop). The design prevents any contact between the needle and the stator and rotor faces.

Note: A detailed discussion on the Installation, Use and Maintenance of the valve is presented in Technical Note 801 from Valco Instruments, Co. Inc. and can be obtained at the Valco website (www.Valco.com)

D.2 Maintenance

In most instances, the only maintenance that is required is cleaning of the valve. Cleaning can often be accomplished by flushing all the lines with an appropriate solvent(s). The selection of the solvent is dependent on the nature of the sample and the mobile phases that are used. Typically solvents such as methanol, acetonitrile, methanol/water (80/20) or acetonitrile/water (80/20) should be used.

D.3 Disassembly/Reassembly of the Valve

Note: Do not disassemble the valve unless system malfunction is definitely isolated to the valve.

D.3.1 Disassembly of the Valve

To disassemble the valve:

a) Use a 9/16" hex driver to remove the socket head screws which secure the cap to the valve (FIGURE D-1)

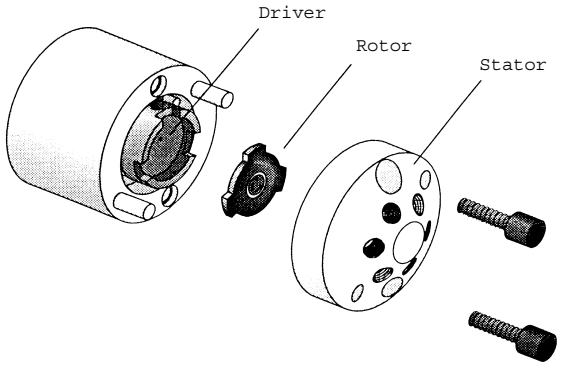

FIGURE D-1. Exploded View of the Valco Model C2 Valve

b) To insure that the sealing surface of the cap is not damaged, rest it on its outer face. If the tubing is still attached, leave it suspended by the tubing.

c) Gently pry the rotor away from the driver with your fingers or a small screwdriver.

d) Examine the rotor sealing surface for scratches.

- If scratches are visible to the naked eye, the rotor must be replaced.
- If no scratches are visible, clean all parts thoroughly with an appropriate solvent. Take care that no surfaces get scratched while you are cleaning the components.

Note: The most common problem in the use of the valve with HPLC is the formation of buffer crystals, which are usually water soluble. After cleaning, it is not necessary to dry the rotor.

D.3.2 Reassembly of the Valve

To reassemble the valve:

a) Replace the rotor in the driver, making sure that the rotor sealing surface with its engraved flow passages is facing out. The pattern is asymmetrical to prevent improper placement.

b) Replace the cap. Insert the two socket head screws and tighten them gently until both are snug.

Caution: Do not overtighten the screws-they simply hold the assembly together and do not affect the sealing force, which is automatically set as the screws close the cap against the valve body.

c) Test the valve by pressurizing the system. If the valve does not hold pressure it should be returned for repair.

Note: When re-installing the valve, make certain that the proper tubing is attached to the appropriate fitting. The loop is connected to ports 1 and 4, the pump is connected at port 2 and the column is connected at port 3.

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APPENDIX E

Digital Inputs and Outputs

E.1 Introduction

The rear panel of the FAMOS™ Well Plate Microautosampler includes a series of I/O connectors that allow the user to interface the microautosampler to other components in their analytical system.

If the microautosampler is used with a LC Packings *UltiMate*™ Capillary and Nano HPLC systems, refer to the User's Manual for that system for specific installation/interfacing information.

E.2 Contact Closure Outputs

I/O Connector P1 (programmable outputs), P4 (marker outputs) and P5 (auxiliary outputs) are contact closure outputs (floating NO/NC contact) as shown in FIGURE E-1.

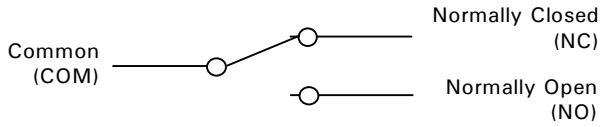
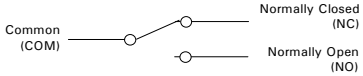


FIGURE E-1. Contact Closure Output

E.2.1 P1 Connector – OUTPUTS

TABLE E-1. P1 Connector Pin Descriptions

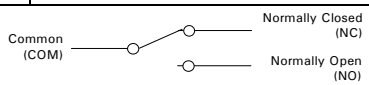
PIN #	Name	Function	PIN #	Name	Function
1	OUT 1	Normally open	7	(Spare)	Normally open
2		Common	8		Common
3		Normally closed	9		Normally closed
4	OUT 2	Normally open	10	Alarm output	Normally open
5		Common	11		Common
6		Normally closed	12		Normally closed
13	24 V DC		14	Power ground	
			15		
			Note: V_{MAX} = 28 V_{DC} / V_{AC}, I_{MAX} = 0.25 A		

Connector P1 OUTPUTS includes 2 programmable outputs and an alarm output

Note: The Alarm output will be activated whenever an error occurs. A description of the error codes of the FAMOS Well Plate Microautosampler is presented in Chapter 6.

E.2.2 P4 Connector – MARKERS

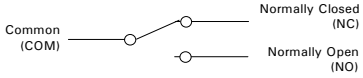
TABLE E-2. P4 Connector Pin Descriptions

PIN #	Name	Function	PIN #	Name	Function
1	Inject Marker	Normally open	7	Labeled Well Marker	Normally open
2		Common	8		Common
3		Normally closed	9		Normally closed
4	Well Marker	Normally open	10	Stop I/O	Normally open
5		Common	11		Common
6		Normally closed	12		Normally closed
13	24 V DC		14	Power ground	
			15		
			Note: V_{MAX} = 28 V_{DC} / V_{AC}, I_{MAX} = 0.25 A		

V_{MAX} = 28 V_{DC}/V_{AC}, I_{MAX} = 0.25 A

E.2.3 P5 Connector – AUXILIARIES

TABLE E-3. P5 Connector Pin Descriptions

PIN #	Name	Function	PIN #	Name	Function
1	AUX 1	Normally open	7	AUX 3	Normally open
2		Common	8		Common
3		Normally closed	9		Normally closed
4	AUX 2	Normally open	10	AUX 4	Normally open
5		Common	11		Common
6		Normally closed	12		Normally closed
13	24 V DC		14	Power ground	
			15		
			Note: V_{MAX} = 28 V_{DC} / V_{AC}, I_{MAX} = 0.25 A		

V_{MAX} = 28 V_{DC}/V_{AC}, I_{MAX} = 0.25 A

E.3 TTL Outputs

The following tables show the marker outputs (P2) and a 4 bit time base code output (P3), programmable in a time base method. Both connectors are TTL level outputs (FIGURE E-2).

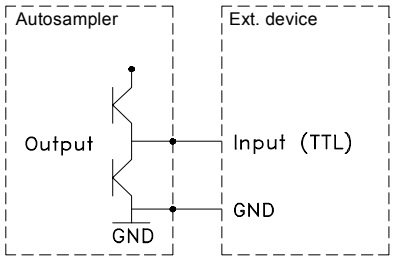


FIGURE E-2. TTL outputs

E.3.1 P2 Connector - TTL OUTPUTS

TABLE E-4. P2 Connector Pin Descriptions

PIN #	Function	PIN #	Function
1	INJECT MARKER	9	not connected
2	VIAL/WELL MARKER	10	not connected
3	LABELED WELL MARKER	11	not connected
4	STOP I/O	12	not connected
5	not connected	13	Signal ground
6	not connected	14	Signal ground
7	not connected	15	Signal ground
8	not connected		
	All markers are active low (logical 0). V_{MAX} = 5.5 V, logical 1 > 3.5 V , logical 0 < 1.0 V. DC output source / sink current ± 20 mA.		

Note: A marker output pulse will be generated when the injection valve switches from LOAD to INJECT. However, in a User Program markers have to be programmed by the user.

E.3.2 P3 Connector - TIMED OUTPUTS

TABLE E-5. P3 Connector Pin Descriptions

Connector P3 TIMED OUTPUTS; 4 bit time base code output

PIN #	Function	PIN #	Function
1	TB 0 (HEX) (1)	6	Signal ground
2	TB 1 (HEX) (2)	7	Signal ground
3	TB 2 (HEX) (4)	8	Signal ground
4	TB 3 (HEX) (8)	9	Signal ground
5	not used		
	All markers are active low (logical 0). V_{MAX} = 5.5 V, logical 1 > 3.5 V , logical 0 < 1.0 V. DC output source / sink current ± 20 mA.		

E.4 TTL Inputs

The TTL input connector (FIGURE E-3) is an active high or active low TTL input; it can be defined in the System Menu. The NEXT INJECTION INPUT and the NEXT WELL INPUT can be used when the FAMOS Well Plate works in REMOTE CONTROL. The FREEZE INPUT and STOP I/O input can be used to control the FAMOS Well Plate by other devices. The four inputs (INPUT 1 to 4) can only be used in the user program, e.g. to control the sequence of the steps in this method. A connection diagram is shown in FIGURE E-3.

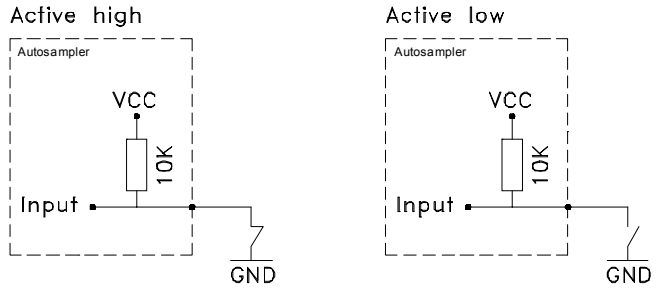


FIGURE E-3. TTL Input

E.4.1 P6 Connector - TTL Inputs

TABLE E-6. P6Connector Pin Descriptions

PIN #	Function	PIN #	Function
1	NEXT INJECTION INPUT	9	Signal ground
2	NEXT WELL INPUT	10	Signal ground
3	FREEZE INPUT	11	Signal ground
4	STOP I/O	12	Signal ground
5	INPUT 1	13	Signal ground
6	INPUT 2	14	Signal ground
7	INPUT 3	15	Signal ground
8	INPUT 4		

Next injection input: This input will start the next injection sequence when the FAMOS Well Plate Microautosampler is started in remote control. When the injection sequence is finished the FAMOS Well Plate Microautosampler will wait for the next input.

From the Ready Menu, a NEXT INJECTION INPUT will start the last programmed series. In this case the FAMOS Well Plate Microautosampler will not wait for the NEXT INJECTION INPUT before continuing with the next injection. The FAMOS Well Plate Microautosampler will execute the complete RUN as if it was started with the Start/Stop key.

Next well input: With this input the FAMOS Well Plate will perform the next injection from the next well, even if not all injections from that well in the programmed injection method have been executed.

Freeze input: The FAMOS Well Plate Microautosampler will freeze the analysis time for the time that this input is active. If the FREEZE INPUT is activated while the analysis time is not running, the FAMOS Well Plate Microautosampler will perform all programmed pre-injection sample handling (mix method and loading part of the injection method). It should be noted that the FAMOS Well Plate Microautosampler will not inject the sample until the FREEZE INPUT is no longer active.

Stop I/O: With this input the run of the FAMOS Well Plate Microautosampler is immediately aborted and the Ready Menu appears in the display. If the FAMOS Well Plate Microautosampler is in remote control, the run of the FAMOS Well Plate Microautosampler is immediately aborted but it remains in remote control and cannot be restarted via a NEXT INJECTION input.

INPUT 1-4: Programmable inputs, can be used in the user program.

APPENDIX F

Programming Forms

The powerful programming features described in Chapter 3 allow the user to set sampling parameters to meet the precise needs of the analyst. On the following pages, we provide blank sheets which can be used for hard copy records of System Menu Settings, Templates, Injection Methods, Wash Methods, Timebase Methods, Mix Methods and User Programs.

In addition, we provide a form which can be used to describe the FAMOS™ Well Plate Microautosampler. This latter form should be faxed to LC Packings when you are enquiring about your system as it includes information that may assist in identifying your unit.

Please feel free to photocopy the forms in this section.

System Menu settings

<GENERAL>			<USAGE>		
Loop volume	....	µL	Protection code	.....	
Needle tubing volume	....	µL	Timebase methods	☐enabled	☐disabled
Syringe volume	....	µL	Mix methods	☐enabled	☐disabled
Syringe speed	....	factor	User program	☐enabled	☐disabled
Needle height	....	mm	User program protection code	.....	
Skip missing vials	☐yes	☐no	Labeled wells	☐enabled	☐disabled
Air segment	☐yes	☐no	Templates	☐enabled	☐disabled
Headspace pressure	☐yes	☐no	Calibration wells	☐enabled	☐disabled
Time display	☐HH:MM:SS ☐H:MM:mm				
Key click	☐on	☐off			
Error beep	☐on	☐off			
Alarm buzzer	☐on	☐off			

<PLATES>		<IO>		
Type:	☐96-low ☐96-high ☐384-low ☐48-vials	Inject-marker pulse length	....	sec
		Well-marker pulse length	....	sec
processing in:	☐ ROWS	Labeled well-marker pulse length	....	sec
	☐ COLUMNS	Input edge next injection	☐falling	☐rising
position first transport vial:	....	Input edge next well	☐falling	☐rising
position last transport vial:	....	Freeze input active	☐low	☐high
		Reset outputs after last series	☐yes	☐no

<CLOCK>	<COMM.>	
☐Off ☐On (yy/mm/dd and hh/mm)	Device identifier:	2....

Comments:

Templates

Template number	Injection method	Mix method	Wash method	Timebase method	User program Y/N	Comments
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						

Comments:

Injection Methods

Injection Method Number	Type a full b partial c pick-up	Anal. Time	Flush Vol.	Inj. / Well	Injection volumes:								
					1	2	3	4	5	6	7	8	9
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
15													
16													
17													
18													
19													
20													
21													
22													
23													
24													

Comments:

Wash Methods

Wash method number	Wash between	Wash volume	Comments:
1	☐injections ☐wells ☐series		
2	☐injections ☐wells ☐series		
3	☐injections ☐wells ☐series		
4	☐injections ☐wells ☐series		
5	☐injections ☐wells ☐series		

Comments:

Timebase Methods

Method number:					
	Action	**At Time**		**Action**	**At Time**
AUX 1	1 AUX-1 ON		ISS-A	1 ISS-A POSITION 6-1	
	1 AUX-1 OFF			1 ISS-A POSITION 1-2	
	2 AUX-1 ON			2 ISS-A POSITION 6-1	
	2 AUX-1 OFF			2 ISS-A POSITION 1-2	
	3 AUX-1 ON			3 ISS-A POSITION 6-1	
	3 AUX-1 OFF			3 ISS-A POSITION 1-2	
	4 AUX-1 ON			4 ISS-A POSITION 6-1	
	4 AUX-1 OFF			4 ISS-A POSITION 1-2	
AUX 2	1 AUX-2 ON		SSV	1 SSV PORT:	
	1 AUX-2 OFF			2 SSV PORT:	
	2 AUX-2 ON			3 SSV PORT:	
	2 AUX-2 OFF			4 SSV PORT:	
	3 AUX-2 ON			5 SSV PORT:	
	3 AUX-2 OFF			6 SSV PORT:	
	4 AUX-2 ON			7 SSV PORT:	
	4 AUX-2 OFF			8 SSV PORT:	
AUX 3	1 AUX-3 ON		CODE	1 CODE-OUT:	
	1 AUX-3 OFF			2 CODE-OUT:	
	2 AUX-3 ON			3 CODE-OUT:	
	2 AUX-3 OFF			4 CODE-OUT:	
	3 AUX 3 ON			5 CODE-OUT:	
	3 AUX 3 OFF			6 CODE-OUT:	
	4 AUX 3 ON			7 CODE-OUT:	
	4 AUX 3 OFF			8 CODE-OUT:	
AUX 4	1 AUX 4 ON				
	1 AUX 4 OFF				
	2 AUX 4 ON				
	3 AUX 4 OFF				
	3 AUX 4 ON				
	3 AUX 4 OFF				
	4 AUX 4 ON				
	4 AUX-4 OFF				
			END	END OF TIMED EVENTS	

Comments:

Mix Method

Method number:

Line	Action	Value	Position	Speed	Height	Line	Action	Value	Position	Speed	Height
1						41					
2						42					
3						43					
4						44					
5						45					
6						46					
7						47					
8						48					
9						49					
10						50					
11						51					
12						52					
13						53					
14						54					
15						55					
16						56					
17						57					
18						58					
19						59					
20						60					
21						61					
22						62					
23						63					
24						64					
25						65					
26						66					
27						67					
28						68					
29						69					
30						70					
31						71					
32						72					
33						73					
34						74					
35						75					
36						76					
37						77					
38						78					
39						79					
40						80					

Comments:

User Program

Line	Action	Value	Position	Speed	Height	Line	Action	Value	Position	Speed	Height
1						41					
2						42					
3						43					
4						44					
5						45					
6						46					
7						47					
8						48					
9						49					
10						50					
11						51					
12						52					
13						53					
14						54					
15						55					
16						56					
17						57					
18						58					
19						59					
20						60					
21						61					
22						62					
23						63					
24						64					
25						65					
26						66					
27						67					
28						68					
29						69					
30						70					
31						71					
32						72					
33						73					
34						74					
35						75					
36						76					
37						77					
38						78					
39						79					
40						80					

Comments:

User Information

Name of user	
Company	
Department	
Address	
Telephone	
Telefax	

FAMOS Well Plate Microautosampler Information

Serial number	
Firmware version	
Purchase date	
Installed options	
Local dealer	
Address	
Telephone	
Fax	
Service engineer	
Address	
Telephone	
Fax	
Comments:	

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APPENDIX G

Connecting the NANO Column (or other Fused Silica Capillary)

G.1 Introduction

These directions should be used when a NANO column or a fused silica capillary is to be connected to the inert (PAEK) injection valve of the FAMOS™ Micro-autosampler or the inert (PAEK) switching valves of the Switchos™ II Advanced Microcolumn Switching Unit.

CAUTION

Caution: Do not use a stainless steel nut and/or ferrule with inert (PAEK) injection/switching valves. The use of stainless steel nuts or ferrules may damage the valve. Use only the supplied fittings (PEEK) and follow the instructions below.

G.2 Using PEEK Fingertights

The PEEK sleeve connection is created using the components shown in FIGURE G-1:

- PEEK fingertight nut,
- PEEK ferrule,
- PEEK sleeve (with an appropriate I.D.),
- NANO column (or any other fused silica capillary)
- The female fitting port of the inert valve
  (FIGURE G-1 shows a stainless steel union, which is used only for the? assembly of the connection).

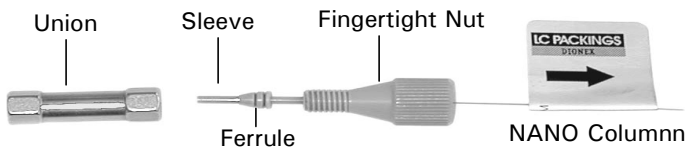

FIGURE G-1 Parts of a PEEK sleeve connection

G.3 Fitting Assembly

To assemble the PEEK sleeve fitting (using the supplied stainless steel union):

a) Slide the fingertight nut and the ferrule onto the sleeve as shown in FIGURE G-1.

b) Insert this assembly into the union that is provided, screwing the nut in two or three turns by hand.

c) Push the sleeve all the way forward into the union so that the sleeve seats firmly. This is essential for a proper zero dead volume connection!

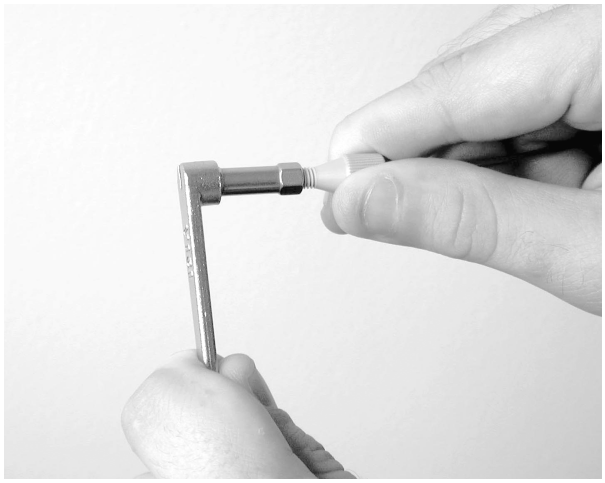

FIGURE G-2 Attaching a PEEK Sleeve connector to a NANO column

d) Manually turn the nut into the union until it is finger tight. Carefully turn the nut 1/4 turn (90°) past the point where the ferrule starts to grab the sleeve (FIGURE G-2). Use a ¼" wrench to retain the union.

e) Remove the pre-assembled sleeve fitting and inspect it. The ferrule should be firmly attached to the tubing (i.e. you should not be able to move it laterally along the tubing axis). If the ferrule is loose, reinstall the tubing in the fitting in the union and tighten it another 1/8 of a turn past finger tight.

f) Push the NANO column (capillary) all the way forward into the pre-assembled sleeve so that the sleeve and column seat firmly. This is essential for a proper zero dead volume connection!

Note: It is essential that the sleeve and the NANO column (capillary) are inserted all the way forward into the union for a proper zero dead volume connection!

g) Manually turn the nut into the union until it is finger tight. Carefully turn the nut 1/4 turn (90°) past the point where the pre-assembled sleeve first start to grab the tubing (FIGURE G-2). Use a ¼" wrench to retain the union.

Note: The amount of force to turn the nut can vary considerably due to the friction between the nut and the threads and as well as the composition and wall thickness of the tubing used. Because of these variables, we do not provide a torque specification.

h) Remove the fitting and inspect it. The PEEK sleeve should be firmly attached to NANO column or the capillary, respectively (i.e. you should not be able to move it laterally along the tubing axis). If the PEEK sleeve is loose, reinstall the fitting in the union and tighten it another 1/8 of a turn past finger tight.

i) Remove, re-inspect, and repeat, if necessary.

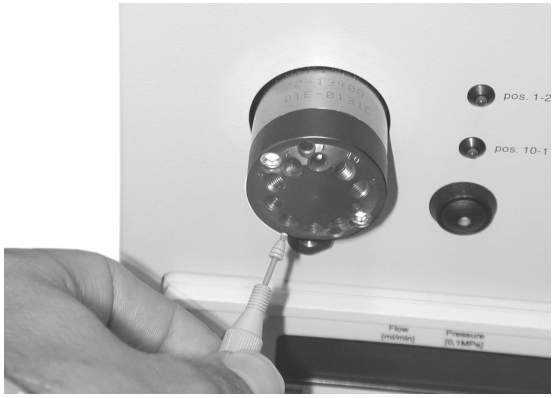

FIGURE G-3 Installing the Pre-assembled Fitting

j) Install the pre-assembled fitting in the inert (PAEK) injection/switching valve and manually turn the nut into the valve until it is finger tight (FIGURE G-3).

CAUTION **Caution: Never use the inert (PAEK) injection/switching valves to pre-assemble a fitting and never use any tool to tighten the fingertight nut. This may lead to a damage of the inert (PAEK) valve.**

G.4 Using Long PEEK Hex Style Nuts

In case the supplied fingertight fittings cannot be used due to limited space, use the long PEEK hex style nuts (P/N 161007) supplied with the instruments instead (FIGURE G-4).

FIGURE G-4 The Long PEEK Hex Style Nut

To use the supplied long hex nuts:

a) Assemble the fitting as described in Section G.3.

b) Install the pre-assembled fitting in the inert (PAEK) injection/switching valve and manually turn the nut into the inert (PAEK) valve until it is finger tight (FIGURE G-3).

c) Carefully tighten the hex style nut using a ¼" wrench.

CAUTION **Caution: Never use the inert (PAEK) injection/switching valves to pre-assemble a fitting and never use any tool to tighten the fingertight nut. This may lead to a damage of the inert (PAEK) valve.**

G.5 Spare Parts Lists

G.5.1 Tubing and Fittings

Part Number	Description
161000	PEEK Fingertight nuts and ferrules for inert injection/switching valves, set of 10
161007	PEEK Hex nuts and ferrules for inert injection/switching valves, set of 10

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Note: Items indicated in Brackets and Capital Letters Refer to Commands on the Display