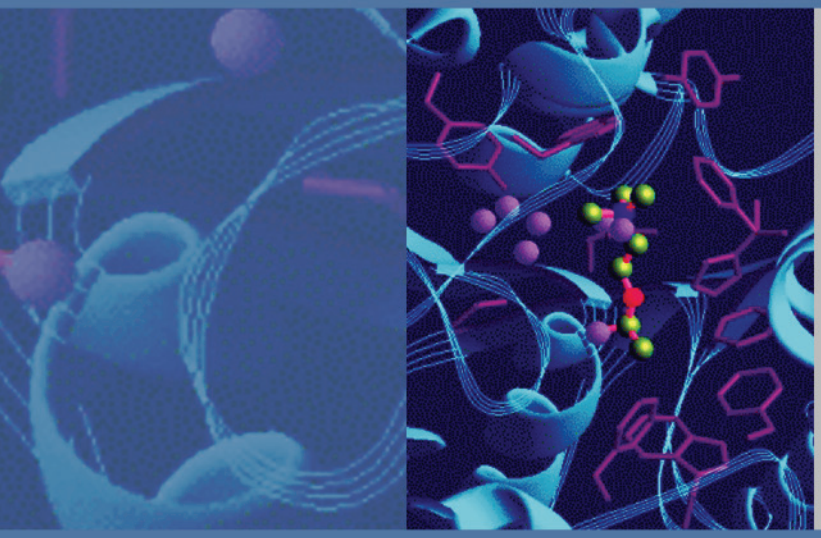

SpectraSYSTEM

AS3000 Autosampler

(with column oven and tray temperature control)

User Guide

A0099-97006 Revision A June 2010

DOCUMENTATION SURVEY

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Release history: Revision A, released June 2010

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Regulatory Compliance

Thermo Fisher Scientific performs complete testing and evaluation of its products to ensure full compliance with applicable domestic and international regulations. When the system is delivered to you, it meets all pertinent electromagnetic compatibility (EMC) and safety standards as described in the next section.

Changes that you make to your system might void compliance with one or more of these EMC and safety standards. Changes to your system include replacing a part or adding components, options, or peripherals not specifically authorized and qualified by Thermo Fisher Scientific. To ensure continued compliance with EMC and safety standards, replacement parts and additional components, options, and peripherals must be ordered from Thermo Fisher Scientific or one of its authorized representatives.

SpectraSYSTEM AS3000 (with oven and tray temperature control)

EMC Directive 2004/108/EC

EMC compliance has been evaluated by TÜV Rheinland of North America.

EN 55011: 2007; A2:2007
EN 61000-3-2: 2006
EN 61000-3-3: 1995, A1: 2001, A2: 2005
EN 61000-4-2: 1995, A1: 1999, A2: 2001
EN 61000-4-3: 2006
EN 61000-4-4: 2004
EN 61000-4-5: 2005
EN 61000-4-6: 2007
EN 61000-4-11: 2004
EN 61326-1: 2006
FCC Class A: CFR 47: Part 15: 2009

Low Voltage Safety Compliance

Low voltage safety compliance has been evaluated by TÜV Rheinland of North America.

This device complies with Low Voltage Directive 2006/95/EC, harmonized standard EN 61010-1: 2001, IEC 61010-1: 2002, UL 61010A-1: 2004, and CAN/CSA 22.2 61010-1: 2004.

Changes that you make to your system may void compliance with one or more of these EMC and safety standards. Changes to your system include replacing a part or adding components, options, or peripherals not specifically authorized and qualified by Thermo Fisher Scientific. To ensure continued compliance with EMC and safety standards, replacement parts and additional components, options, and peripherals must be ordered from Thermo Fisher Scientific or one of its authorized representatives.

FCC Compliance Statement

THIS DEVICE COMPLIES WITH PART 15 OF THE FCC RULES. OPERATION IS SUBJECT TO THE FOLLOWING TWO CONDITIONS: (1) THIS DEVICE MAY NOT CAUSE HARMFUL INTERFERENCE, AND (2) THIS DEVICE MUST ACCEPT ANY INTERFERENCE RECEIVED, INCLUDING INTERFERENCE THAT MAY CAUSE UNDESIRED OPERATION.

CAUTION Read and understand the various precautionary notes, signs, and symbols contained inside this manual pertaining to the safe use and operation of this product before using the device.

Notice on Lifting and Handling of Thermo Scientific Instruments

For your safety, and in compliance with international regulations, the physical handling of this Thermo Fisher Scientific instrument ***requires a team effort*** to lift and/or move the instrument. This instrument is too bulky for one person alone to lift or move safely.

Notice on the Proper Use of Thermo Scientific Instruments

In compliance with international regulations: Use of this instrument in a manner not specified by Thermo Fisher Scientific could impair any protection provided by the instrument.

Notice on the Susceptibility to Electromagnetic Transmissions

Your instrument is designed to work in a controlled electromagnetic environment. Do not use radio frequency transmitters, such as mobile phones, in close proximity to the instrument.

For manufacturing location, see the label on the instrument.

WEEE Compliance

This product is required to comply with the European Union's Waste Electrical & Electronic Equipment (WEEE) Directive 2002/96/EC. It is marked with the following symbol:

Thermo Fisher Scientific has contracted with one or more recycling or disposal companies in each European Union (EU) Member State, and these companies should dispose of or recycle this product. See www.thermo.com/WEEERoHS for further information on Thermo Fisher Scientific's compliance with these Directives and the recyclers in your country.

WEEE Konformität

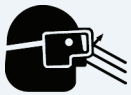
Dieses Produkt muss die EU Waste Electrical & Electronic Equipment (WEEE) Richtlinie 2002/96/EC erfüllen. Das Produkt ist durch folgendes Symbol gekennzeichnet:

Thermo Fisher Scientific hat Vereinbarungen mit Verwertungs-/Entsorgungsfirmen in allen EU-Mitgliedsstaaten getroffen, damit dieses Produkt durch diese Firmen wiederverwertet oder entsorgt werden kann. Mehr Information über die Einhaltung dieser Anweisungen durch Thermo Fisher Scientific, über die Verwerter, und weitere Hinweise, die nützlich sind, um die Produkte zu identifizieren, die unter diese RoHS Anweisung fallen, finden sie unter www.thermo.com/WEEERoHS.

Conformité DEEE

Ce produit doit être conforme à la directive européenne (2002/96/EC) des Déchets d'Equipements Electriques et Electroniques (DEEE). Il est marqué par le symbole suivant:

Thermo Fisher Scientific s'est associé avec une ou plusieurs compagnies de recyclage dans chaque état membre de l'union européenne et ce produit devrait être collecté ou recyclé par celles-ci. Davantage d'informations sur la conformité de Thermo Fisher Scientific à ces directives, les recycleurs dans votre pays et les informations sur les produits Thermo Fisher Scientific qui peuvent aider la détection des substances sujettes à la directive RoHS sont disponibles sur www.thermo.com/WEEERoHS.

CAUTION Symbol	CAUTION	VORSICHT	ATTENTION	PRECAUCION	AVVERTENZA
	**Electric Shock:** This instrument uses high voltages that can cause personal injury. Before servicing, shut down the instrument and disconnect the instrument from line power. Keep the top cover on while operating the instrument. Do not remove protective covers from PCBs.	**Elektroschock:** In diesem Gerät werden Hochspannungen verwendet, die Verletzungen verursachen können. Vor Wartungsarbeiten muß das Gerät abgeschaltet und vom Netz getrennt werden. Betreiben Sie Wartungsarbeiten nicht mit abgenommenem Deckel. Nehmen Sie die Schutzabdeckung von Leiterplatten nicht ab.	**Choc électrique:** L'instrument utilise des tensions capables d'infliger des blessures corporelles. L'instrument doit être arrêté et débranché de la source de courant avant tout intervention. Ne pas utiliser l'instrument sans son couvercle. Ne pas enlever les étuis protecteurs des cartes de circuits imprimés.	**Descarga eléctrica:** Este instrumento utiliza altas tensiones, capaces de producir lesiones personales. Antes de dar servicio de mantenimiento al instrumento, éste debera apagarse y desconectarse de la línea de alimentacion eléctrica. No opere el instrumento sin sus cubiertas exteriores quitadas. No remueva las cubiertas protectoras de las tarjetas de circuito impreso.	**Shock da folgorazione.** L'apparecchio è alimentato da corrente ad alta tensione che puo provocare lesioni fisiche. Prima di effettuare qualsiasi intervento di manutenzione occorre spegnere ed isolare l'apparecchio dalla linea elettrica. Non attivare lo strumento senza lo schermo superiore. Non togliere i coperchi a protezione dalle schede di circuito stampato (PCB).
	**Chemical:** This instrument might contain hazardous chemicals. Wear gloves when handling toxic, carcinogenic, mutagenic, or corrosive or irritant chemicals. Use approved containers and proper procedures to dispose waste oil.	**Chemikalien:** Dieses Gerät kann gefährliche Chemikalien enthalten. Tragen Sie Schutzhandschuhe beim Umgang mit toxischen, karzinogenen, mutagenen oder ätzenden/reizenden Chemikalien. Entsorgen Sie verbrauchtes Öl entsprechend den Vorschriften in den vorgeschriebenen Behältern.	**Chimique:** Des produits chimiques dangereux peuvent se trouver dans l'instrument. Portez des gants pour manipuler tous produits chimiques toxiques, cancérigènes, mutagènes, ou corrosifs/irritants. Utiliser des récipients et des procédures homologuées pour se débarrasser des déchets d'huile.	**Química:** El instrumento puede contener productos quimicos peligrosos. Utilice guantes al manejar productos quimicos tóxicos, carcinogenos, mutagenos o corrosivos/irritantes. Utilice recipientes y procedimientos aprobados para deshacerse del aceite usado.	**Prodotti chimici.** Possibile presenza di sostanze chimiche pericolose nell'apparecchio. Indossare dei guanti per maneggiare prodotti chimici tossici, cancerogeni, mutageni, o corrosivi/irritanti. Utilizzare contenitori aprovo e seguire la procedura indicata per lo smaltimento dei residui di olio.
	**Heat:** Before servicing the instrument, allow any heated components to cool.	**Hitze:** Warten Sie erhitzte Komponenten erst nachdem diese sich abgekühlt haben.	**Haute Temperature:** Permettre aux composants chauffés de refroidir avant tout intervention.	**Altas temperaturas:** Permita que lop componentes se enfríen, ante de efectuar servicio de mantenimiento.	**Calore.** Attendere che i componenti riscaldati si raffreddino prima di effetturare l'intervento di manutenzione.
	**Fire:** Use care when operating the system in the presence of flammable gases.	**Feuer:** Beachten Sie die einschlägigen VorsichtsmaBnahmen, wenn Sie das System in Gegenwart von entzündbaren Gasen betreiben.	**Incendie:** Agir avec précaution lors de l'utilisation du système en présence de gaz inflammables.	**Fuego:** Tenga cuidado al operar el sistema en presencia de gases inflamables.	**Incendio.** Adottare le dovute precauzioni quando si usa il sistema in presenza di gas infiammabili.
	**Eye Hazard:** Eye damage could occur from splattered chemicals or flying particles. Wear safety glasses when handling chemicals or servicing the instrument.	**Verletzungsgefahr der Augen:** Verspritzte Chemikalien oder kleine Partikel können Augenverletzungen verursachen. Tragen Sie beim Umgang mit Chemikalien oder bei der Wartung des Gerätes eine Schutzbrille.	**Danger pour les yeux:** Des projections chimiques, liquides, ou solides peuvent être dangereuses pour les yeux. Porter des lunettes de protection lors de toute manipulation de produit chimique ou pour toute intervention sur l'instrument.	**Peligro par los ojos:** Las salicaduras de productos químicos o particulas que salten bruscamente pueden causar lesiones en los ojos. Utilice anteojos protectores al mnipular productos químicos o al darle servicio de mantenimiento al instrumento.	**Pericolo per la vista.** Gli schizzi di prodotti chimici o delle particelle presenti nell'aria potrebbero causare danni alla vista. Indossare occhiali protettivi quando si maneggiano prodotti chimici o si effettuano interventi di manutenzione sull'apparecchio.
	**General Hazard:** A hazard is present that is not included in the above categories. Also, this symbol appears on the instrument to refer the user to instructions in this manual.	**Allgemeine Gefahr:** Es besteht eine weitere Gefahr, die nicht in den vorstehenden Kategorien beschrieben ist. Dieses Symbol wird im Handbuch auBerdem dazu verwendet, um den Benutzer auf Anweisungen hinzuweisen.	**Danger général:** Indique la présence d'un risque n'appartenant pas aux catégories citées plus haut. Ce symbole figure également sur l'instrument pour renvoyer l'utilisateur aux instructions du présent manuel.	**Peligro general:** Significa que existe un peligro no incluido en las categorias anteriores. Este simbolo también se utiliza en el instrumento par referir al usuario a las instrucciones contenidas en este manual.	**Pericolo generico.** Pericolo non compreso tra le precedenti categorie. Questo simbolo è utilizzato inoltre sull'apparecchio per segnalare all'utente di consultare le istruzioni descritte nel presente manuale.
	When the safety of a procedure is questionable, contact your local Technical Support organization for Thermo Fisher Scientific San Jose Products.	Wenn Sie sich über die Sicherheit eines Verfahrens im unklaren sind, setzen Sie sich, bevor Sie fortfahren, mit Ihrer lokalen technischen Unterstützungsorganisation für Thermo Fisher Scientific San Jose Produkte in Verbindung.	Si la sûreté d'une procédure est incertaine, avant de continuer, contacter le plus proche Service Clientèle pour les produits de Thermo Fisher Scientific San Jose.	Cuando la certidumbre acerca de un procedimiento sea dudosa, antes de proseguir, pongase en contacto con la Oficina de Asistencia Tecnica local para los productos de Thermo Fisher Scientific San Jose.	Quando e in dubbio la misura di sicurezza per una procedura, prima di continuare, si prega di mettersi in contatto con il Servizio di Assistenza Tecnica locale per i prodotti di Thermo Fisher Scientific San Jose.

CAUTION Symbol	CAUTION	危険警告	危險警告
	**Electric Shock:** This instrument uses high voltages that can cause personal injury. Before servicing, shut down the instrument and disconnect the instrument from line power. Keep the top cover on while operating the instrument. Do not remove protective covers from PCBs.	**電撃：**この計測器は高電圧を使用し、人体に危害を与える可能性があります。保守・修理は、必ず操業を停止し、電源を切ってから実施して下さい。上部カバーを外したままで 計測器を使用しないで下さい。プリント配線板の保護カバーは外さないで下さい。	電擊：儀器設備使用會造成人身傷害的高伏電壓。在維修之前，必須先關儀器設備並切除電源。務必要在頂蓋蓋上的情況下操作儀器。請勿拆除PCB保護蓋。
	**Chemical:** This instrument might contain hazardous chemicals. Wear gloves when handling toxic, carcinogenic, mutagenic, or corrosive or irritant chemicals. Use approved containers and proper procedures to dispose waste oil.	**化学物質：**危険な化学物質が計測器中に存在している可能性があります。毒性、発がん性、突然変異性、腐食・刺激性などのある薬品を取り扱う際は、手袋を着用して下さい。廃油の処分には、規定の容器と手順を使用して下さい。	化學品：儀器設備中可能存在有危險性的化學物品。接觸毒性致癌、誘變或腐蝕／刺激性化學品時，請配帶手套。處置廢油時，請使用經過許可的容器和程序。
	**Heat:** Before servicing the instrument, allow any heated components to cool.	**熱：**熱くなった部品は冷えるのを待ってから保守・修理を行って下さい。	高溫：請先等高溫零件冷卻之後再進行維修。
	**Fire:** Use care when operating the system in the presence of flammable gases.	**火災：**可燃性のガスが存在する場所でシステムを操作する場合は、充分な注意を払って下さい。	火災：在有易燃氣體的場地操作該系統時，請務必小心謹慎。
	**Eye Hazard:** Eye damage could occur from splattered chemicals or flying particles. Wear safety glasses when handling chemicals or servicing the instrument.	**眼に対する危険：**化学物質や微粒子が飛散して眼を傷つける危険性があります。化学物質の取り扱い、あるいは計測器の保守・修理に際しては防護眼鏡を着用して下さい。	眼睛傷害危險：飛濺的化學品或顆粒可能造成眼睛傷害。處理化學品或維儀器設備時請佩戴安全眼鏡。
	**General Hazard:** A hazard is present that is not included in the above categories. Also, this symbol appears on the instrument to refer the user to instructions in this manual.	**一般的な危険：**この標識は上記以外のタイプの危険が存在することを示します。また、計測器にこの標識がついている場合は、本マニュアル中の指示を参照して下さい。	一般性危險：說明未包括在上述類別中的其他危險。此外，儀器設備上使用這個標誌，以指示用戶本使用手冊中的說明。
	When the safety of a procedure is questionable, contact your local Technical Support organization for Thermo Fisher Scientific San Jose Products.	**安全を確保する手順がよくわからない時は、作業を一時中止し、お近くのサーモエレクトロンサンローゼプロダクトのテクニカールサポートセンターごご連絡ください。**	如对安全程序有疑问，请在操作之前与当地的菲尼根技术服务中心联系。

Contents

Preface

This guide describes how to install, operate, and maintain the SpectraSYSTEM™ AS3000 autosampler, as well as how to inject samples and perform diagnostics from the front panel keypad.

Contents

Safety and Special Notices

This guide contains the following safety and special notices.

CAUTION Alerts you to situations that could result in personal injury and tells you how to avoid them.

CAUTION High Voltage. Alerts you to the presence of high voltage and to the potential injury that could occur from electrical shock if you came into contact with a specific instrument area or component. Also indicates how to avoid contact with the high-voltage areas in your instrument.

CAUTION Hot Surface. Alerts you to potential injury that could occur from contact with a heated surface or area on or in an instrument. Also, tells you how to avoid contact with the heated surfaces in your instrument.

CAUTION Alerts you to situations that could result in personal injury caused by lifting objects that are too heavy, too bulky, or both.

IMPORTANT Alerts you to connections to the building ground.

IMPORTANT Alerts you to grounding connections that require a protective earth ground.

IMPORTANT Alerts you to the correct operating or maintenance procedures needed to prevent equipment or data damage. It also alerts you to important exceptions, side effects, or unexpected occurrences that can result from certain actions.

Tip Calls out general rules or shortcuts. Tips specify ways to obtain the best performance and results from your instrument.

Safety Precautions for the SpectraSYSTEM Autosampler

Follow these safety precautions for the SpectraSYSTEM autosampler.

Grounding Requirements

The SpectraSYSTEM autosampler requires two levels of grounding. When you install the SpectraSYSTEM autosampler, connect the power cord to a power outlet with a protective earth ground. In addition, connect the grounding post on the back panel of the autosampler to the building ground (see "Connecting the Autosampler to the Building Ground" on page 19).

IMPORTANT Make sure that the power cord is connected to a power outlet with a protective earth ground.

IMPORTANT Make sure that the grounding post on the back panel of the autosampler is connected to the building ground.

High-Temperature Components

When you set the column oven to high temperatures, the compartment's metal components can cause burns. When you want to change the LC column or tighten the liquid connections to the LC column, turn off the column oven, and wait for the oven to cool to room temperature.

CAUTION Never touch the metal components of the column oven compartment when you are operating the oven at high temperatures. The metal components can cause burns.

Heavy Lifting Hazard

Because the autosampler is bulky and heavy, lifting it requires a team effort. For information on how to safely lift the autosampler, see "Lifting the Autosampler" on page 17.

CAUTION For your safety, and in compliance with international regulations, ask for assistance when moving the autosampler from one benchtop location to another. The autosampler is too bulky for one person alone to lift or move safely.

Good Laboratory Practices

To obtain optimal performance from your LC system and to prevent personal injury or injury to the environment, do the following:

- Keep good records.
- Read the manufacturers' Material Safety Data Sheets (MSDSs) for the chemicals being used in your laboratory.
- Remove particulate matter from your samples before you inject them into the liquid chromatograph.
- Use HPLC-grade solvents.
- Connect the drainage tubes from the pump, autosampler, and detector to an appropriate waste receptacle. Dispose of solvents as specified by local regulations.

Keeping Good Records

To help identify and isolate problems with either your equipment or your methodology, keep good records of all system conditions (for example,% RSDs on retention times and peak areas, peak shape, and resolution). At a minimum, keep a chromatogram of a typical sample and standard mixture, well documented with system conditions, for future reference. Careful comparison of retention times, peak shapes, peak sensitivity, and baseline noise can provide valuable clues to identifying and solving future problems.

Chemical Toxicity

Although the large volume of toxic and flammable solvents used and stored in laboratories can be quite dangerous, do not ignore the potential hazards posed by your samples. Take special care to read and follow all precautions that ensure proper ventilation, storage, handling, and disposal of both solvents and samples. Become familiar with the toxicity data and potential hazards associated with all chemicals by referring to the manufacturers' MSDSs.

Sample Preparation

Always consider the solubility of your sample in the solvent/mobile phase. Sample precipitation can plug the column, tubing, or flowcell causing flow restriction. This obstruction can result in irreparable damage to the system. To avoid damage caused by particulate matter, filter samples through 0.45 or 0.2 micron (or less) filters.

Solvent Requirements

Many chemical manufacturers provide a line of high-purity or HPLC-grade reagents that are free of chemical impurities. Routine filtration of all solvents or eluents through a 0.45 or 0.2 micron (or less) fluorocarbon filter before placing them in the solvent reservoir significantly prolongs the life and effectiveness of the inlet filters, check valves and seals, injection valve, and column. Typically, HPLC-grade solvents do not require filtration.

Choose a mobile phase that is compatible with the sample and column you have selected for your separation. Remember that some solvents are corrosive to stainless steel.

Solvent Disposal

Make sure you have a solvent waste container or other kind of drain system available at or below the benchtop level. Most solvents have special disposal requirements and should not be disposed of directly down a drain. Follow all governmental regulations when disposing of any chemical.

High-Pressure Systems and Leaks

LC systems operate at high pressures. Because liquids are not highly compressible, they do not store much energy. Accordingly, there is little immediate danger from the high pressures in an LC system. However, if a leak occurs, correct it as soon as possible. Always wear eye and skin protection when operating or maintaining an LC system. Always shut down the system and return it to atmospheric pressure before attempting any maintenance.

Contacting Us

There are several ways to contact Thermo Fisher Scientific for the information you need.

❖ To contact Technical Support

Phone	800-532-4752
Fax	561-688-8736
E-mail	us.techsupport.analyze@thermofisher.com
Knowledge base	www.thermokb.com

Find software updates and utilities to download at mssupport.thermo.com.

❖ To contact Customer Service for ordering information

Phone	800-532-4752
Fax	561-688-8731
E-mail	us.customer-support.analyze@thermofisher.com
Web site	www.thermo.com/ms

❖ To copy manuals from the Internet

Go to http://sjsupport.thermofinnigan.com/public/index_download_customer.asp and click **Customer Manuals** in the left margin of the window.

❖ To suggest changes to documentation or to Help

- To provide us with comments about this document, click the link below. Thank you in advance for your help.

- Send an e-mail message to the Technical Publications Editor at techpubs-lcms@thermofisher.com.

Introduction

This chapter introduces the Thermo Scientific SpectraSYSTEM AS3000 autosampler (see Figure 1) and describes the conventions used in this manual.

Figure 1. SpectraSYSTEM AS3000 autosampler

Contents

Functional Description

These topics describe the autosampler's components and injection types:

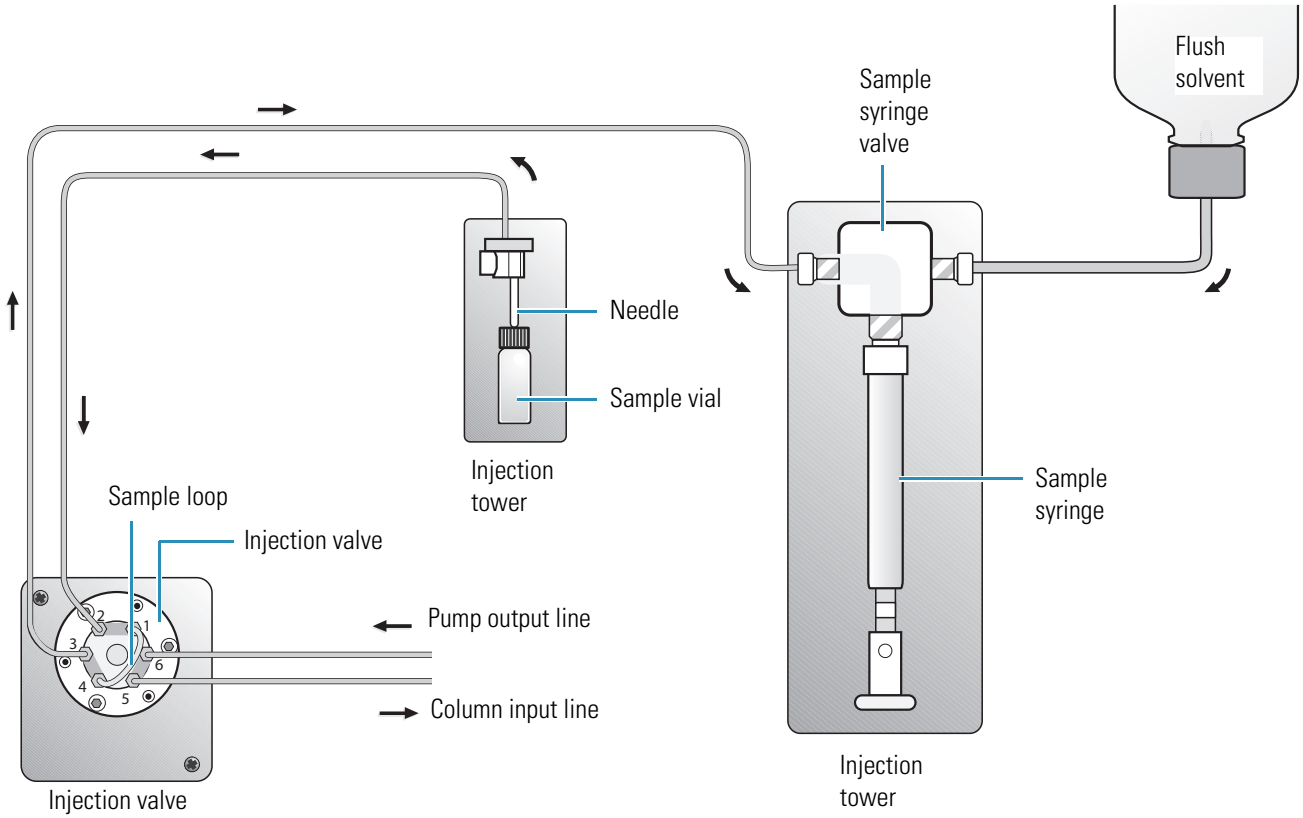
Autosampler Components

The autosampler consists of the following components:

- Injection valve and sample loop
- XYZ arm with holding hook and needle
- Syringe and two-position syringe valve
- Tray compartment and temperature control unit
- Column oven compartment

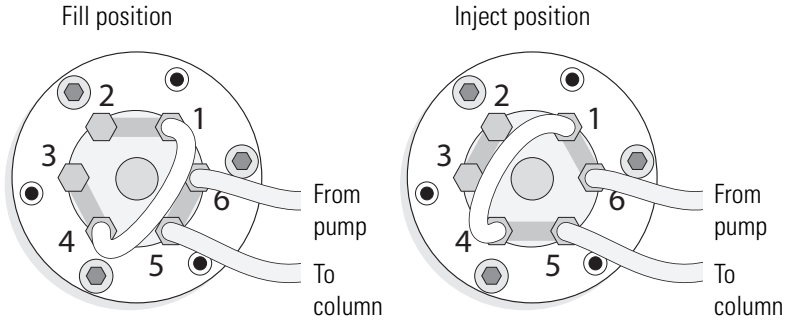
Figure 2 shows the components of the autosampler's injection system.

Figure 2. Injection system



Prior to sample analysis, the operator loads vials filled with samples and calibration standards into three trays that hold 35 vials each if the tray temperature control unit is installed. During operation, the autosampler's mechanical arm (the XYZ arm) locates the requested vial. The hook on the end of the arm moves under the selected vial and lifts it from the sample tray. The XYZ arm transports the vial to the sample tower and the sample needle for sample injection. As necessary, the injection valve switches between the inject and fill positions (see Figure 3) to draw sample or solvent into the autosampler lines and to inject sample onto the column.

Figure 3. Inject and fill positions for the injection valve



The tray compartment holds the sample trays, the injection tower, and the sample syringe. The temperature control unit for the tray compartment consists of four Peltier devices, two high-speed fans, and a heat sink. Heat is pumped from the sample compartment into the heat sink and then blown out the back of the instrument by the two fans. A temperature sensor sends the temperature reading back to the microprocessor, which controls the operation of the four Peltier devices to regulate the sample compartment temperature. Similar to the column oven, the tray temperature control unit is a closed assembly and requires no routine maintenance.

Note The current version of the SpectraSYSTEM AS3000 autosampler includes a temperature-control unit for the tray compartment and the vial trays hold 35 vials each.

The temperature-control unit maintains the temperature of the tray compartment within ± 2 °C. At ambient room temperature, the tray compartment takes approximately 30 minutes to reach a temperature of 0 °C and approximately 10 minutes to reach a temperature of 60 °C.

The actual temperature inside the autosampler vial depends on the specified tray temperature, type of vial, sample buffer, and so on. Compared to the tray temperature, the vial temperature tends to be higher below 30 °C and lower above 30 °C. The time lag in heating and cooling is due to variations in ambient temperatures, indirect contact with temperature control units, and vial type.

The column oven compartment holds the LC column. The column oven contains a resistive thermal device that heats up to stabilize the column temperature. A temperature sensor detects the temperature and sends the temperature reading back to the microprocessor, which turns the resistive device on or off to regulate the temperature. This assembly is closed and requires no routine maintenance.

Injection Types

The SpectraSYSTEM AS3000 autosampler has three injection types:

- Full-loop Injection Type
- Pull-loop Injection Type
- PushLoop Injection Type

The pull-loop and PushLoop™ injection types are partial-loop injection types that allow you to inject variable amounts of sample.

Full-loop Injection Type

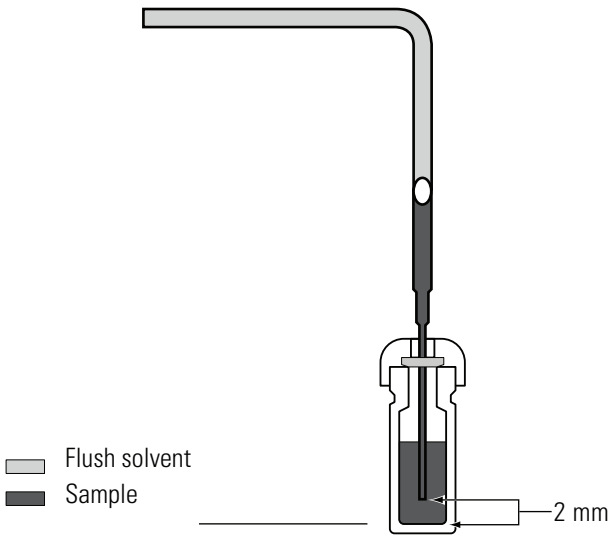
Use the full-loop injection type when you need the highest possible injection-to-injection precision and you have sufficient sample to overfill the sample loop. During a full-loop injection, the autosampler overfills the sample loop, and the mobile phase from the pump backflushes the contents of the sample loop onto the LC column.

During a full-loop injection, the autosampler performs these tasks:

1. Picks up the sample vial from the vial position that you specify in the acquisition sequence, and transports the vial to the injection tower.
2. Pushes the sample needle through the vial septum to the depth specified in the method (Needle Height from Bottom parameter).

   Figure 4 shows the autosampler needle descending to a depth of 2 mm from the bottom of the sample vial.

Figure 4. Needle height from bottom (of the vial)



3. Switches the injection valve to the fill position.
4. Withdraws sample solution from the vial and overfills the sample loop.

   The speed at which the autosampler withdraws sample depends on the sample viscosity that you specify in the instrument method (Normal, Medium, or High). The amount of sample withdrawn depends on the injection volume that you specify in the acquisition sequence.

   The autosampler uses the following algorithm to overfill the sample loop:

   Volume withdrawn = Requested injection volume × 1.33 + 70 µL
5. Switches the injection valve to the inject position, allowing the mobile phase to backflush the sample out of the loop and through the LC column.
6. Flushes the needle tubing and needle with flush solvent from the flush bottle. You specify the flush volume in the instrument method. The default flush volume is 400 µL.

Pull-loop Injection Type

Use the pull-loop injection type when you have a limited amount of sample. During a pull-loop injection, the autosampler pulls only the requested sample volume into the sample loop.

Because dissolved gases can outgas as the autosampler pulls the sample through the needle tubing, the pull-loop injection type is less precise than the Pushloop injection type. The bubble formation within the sample slug decreases the reproducibility of the sample volume pulled into the sample loop.

During a pull-loop injection, the autosampler performs the following tasks:

1. Draws an air bubble into the needle to isolate the incoming sample slug from the flush solvent already present in the lines.
2. Switches the injection valve to the fill position.
3. Lowers the needle into the sample vial.
4. Draws sample into the needle tubing.
5. Raises the needle out of the vial.
6. Draws air into the needle tubing as it pulls the sample solution into the sample loop.
7. Switches the injection valve to the inject position, allowing the mobile phase from the LC pump to backflush the sample from the sample loop onto the LC column.

PushLoop Injection Type

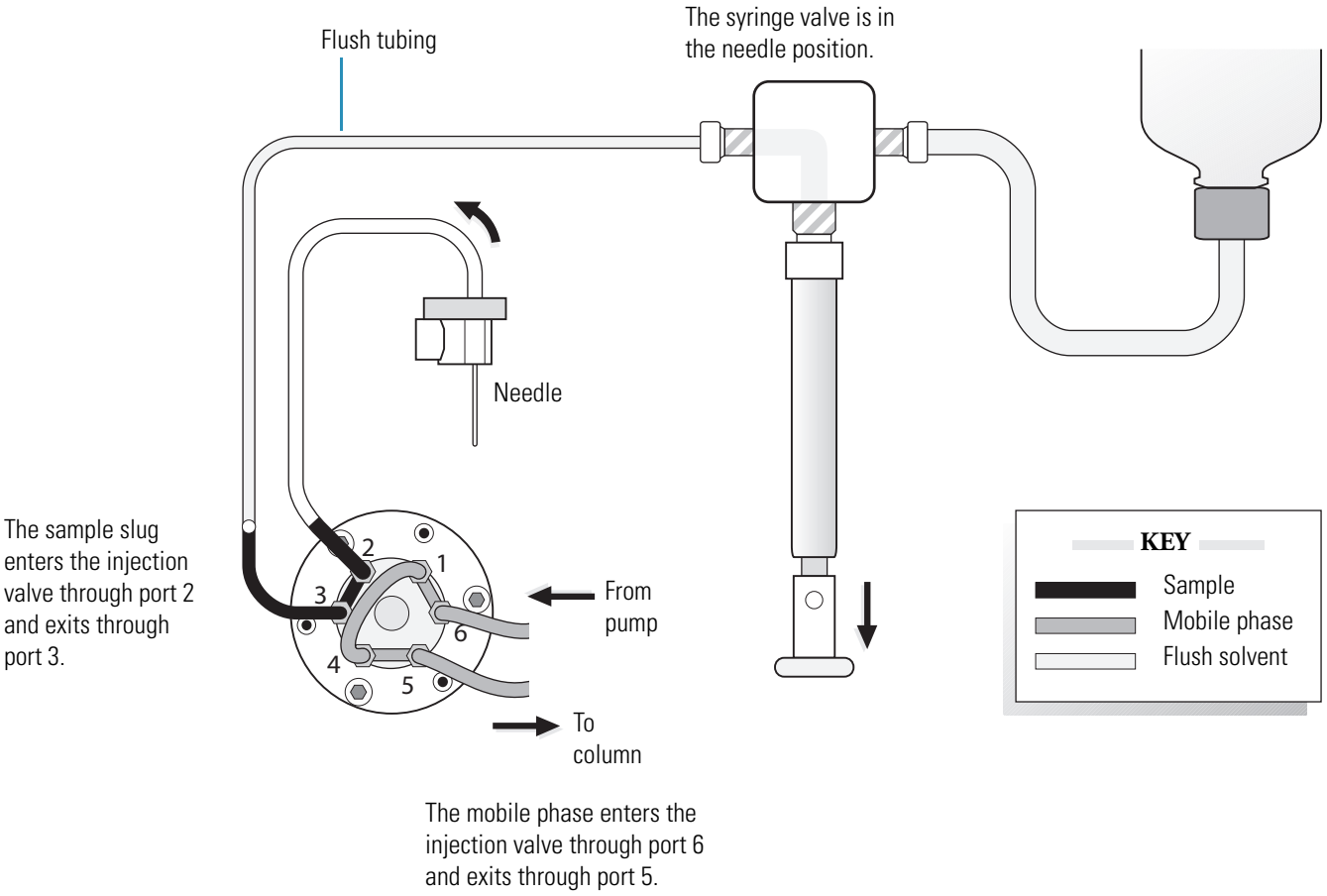
Use the PushLoop injection type when you want to vary the injection volume and injection-to-injection precision is important.

During a PushLoop injection, the autosampler performs these tasks:

1. Draws sample into the needle tubing and through the injection valve as shown in Figure 5:
   a. Switches the injection valve to the inject position.
   b. Draws an air bubble into the needle.
   c. Lowers the needle into the sample vial.
   d. Draws sample into the needle tubing.
   e. Draws a portion of the sample solution through the injection valve.

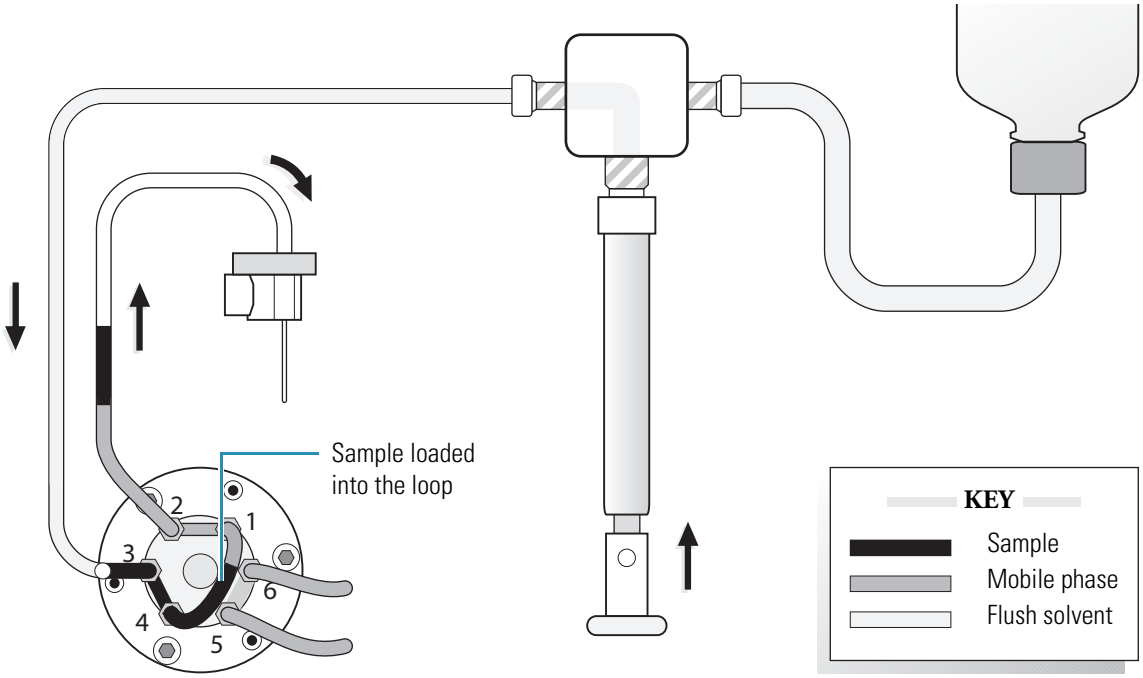
      This step flushes the solvent path between the needle tubing and the sample loop with the sample solution.

Figure 5. PushLoop injection—step 1



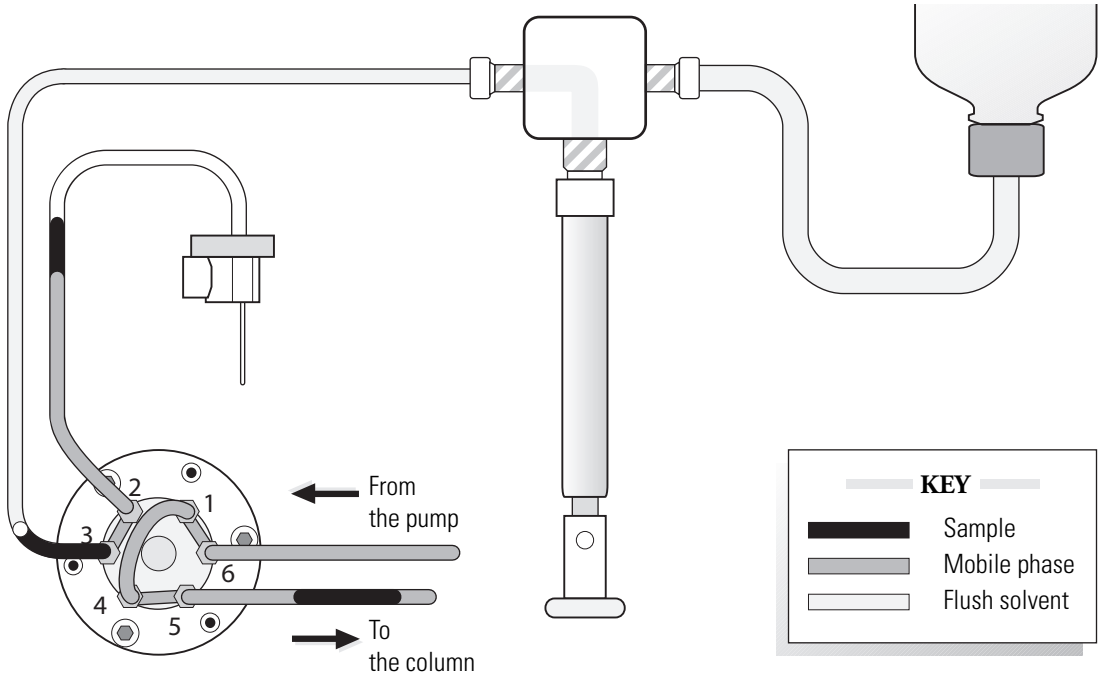
2. Loads the sample loop with the requested injection volume as follows (see Figure 6):
   a. Switches the injection valve to the fill position.
   b. Pushes the requested injection volume into the sample loop.

Figure 6. PushLoop injection—step 2



3. Switches the injection valve to the inject position. Mobile phase enters the injection valve through port 6, backflushing the sample out of the loop and onto the column (see Figure 7).

Figure 7. PushLoop injection—step 3



Operation Modes

The SpectraSYSTEM autosampler has two modes of operation: Stand Alone and SpectraNet. In the Stand Alone mode, you control the autosampler from its keypad controls and acquire data to an external device, such as an integrator. In the SpectraNet mode, the SN4000 system controller controls the SpectraSYSTEM HPLC modules through SpectraNET™ communication. The SN4000 system controller transmits information from the system modules, such as the autosampler's tray and column oven compartment temperatures, the system pressure monitored by the pump, and the data stream from the detector to the data system computer.

For information on setting up the system for SpectraNET control, see "SpectraNet Mode" on page 31. For information on controlling the autosampler from the data system, refer to the Help and manuals provided with the data system.

For information on setting up the system for stand-alone control, see "Stand Alone Mode" on page 39. For information on controlling the autosampler from its command center, see "Standalone Operation from the Command Center" on page 63.

Manual Conventions

This manual uses several conventions for working with the autosampler keys and menu displays.

Autosampler Displays

The autosampler provides a two-line display as shown in the next figure. For manual illustrations of the autosampler menu, the triangular cursor location is indicated by a caret (>).

- A two-line menu display

```
>FILES              QUEUE                    TESTS
          COMMANDS              OPTIONS
```

  Frequently, the two lines shown on the display are only part of a longer menu that you can see when you press the instrument's DOWN ARROW key (▼).

  In this manual, menus having more than two lines are represented with dashed lines.

- A menu longer than two lines

```
Configurations                        1
Ready Participation
------------------------------------------------------
Input Polarity
Output Polarity
------------------------------------------------------
                                      2
 Shutdown
 Maintenance Log
```

Text Conventions

This manual uses typographic conventions to differentiate among keys, menus, and fields.

Keys

The manual indicates text keys on the autosampler in all-capital letters, with the exception of the Samples key. For example: Press the MENU key.

Menus

Use of bold text and the right-arrow key, ***Menu name*** **>** ***Menu command***, indicate instrument display menu selections. For example: From the main menu, select **Files > Load**.

Fields

To indicate field names as shown on the instrument display, this manual uses capitalization. Generally, the first letters of field names are capitalized. For example, in the Samples menu, go to the Injection Volume field.

Standard Words

For this manual, the words *select* and *enter* specifically refer to the autosampler menu commands and value fields.

Select

The word *select* is used when you must choose from among available options. For example, to select a particular menu option, you move the cursor to the appropriate selection and press the ENTER key. To select a field entry, move the cursor to the appropriate field, and then press the [+] and [–] keys to scroll to the desired value.

Enter

The word *enter* is used when you must specify individual alphanumeric digits. To enter a particular value, move the cursor to the field and press the [+] and [–] keys to increment or decrement each digit in the field until the desired value or letter appears.

Specifications

These are the specifications for the SpectraSYSTEM AS3000 autosampler.

Vial capacity:	105 in three removable trays
	Note For earlier versions of the autosampler, the tray temperature control unit was a hardware option. The tray compartment for autosamplers that do not have this option installed hold 120 vials in three removable trays.
Variable-volume:	
Injection precision	<0.5% RSD @ 10 µL or greater
Injection volume	• 0.1 to 100 µL injection standard (250 µL syringe) • Up to 1000 µL injections with larger loops and syringes
Fixed-loop:	
Injection precision	<0.5% RSD @ 10 µL or greater
Injection volume	• 20 µL standard (250 µL syringe) • 1000 µL injections with larger loops and syringes
Sample carryover:	Typically <0.01% at 400 µL flush volume
	Note The flush volume is user-settable from 0 to 5000 µL.
Minimum sample volume:	1 µL can be injected from a sample volume of 10 µL with a standard needle
Needle height:	Settable in 0.1 mm increments from 0 to 20 mm
Column oven (optional):	• Settable in 1° increments from 20 to 80 °C, starting at 5 °C above ambient • Temperature stability typically ±0.2 °C • Accommodates up to 30 cm columns
Method files:	• Protected in non-volatile memory
Communications:	• Pump Ready • Inject Hold
Outputs:	• Pump Stop • Autosampler Ready • Inject • Gradient Start • Four timed-event outputs • BCD (optional) • SpectraNET/RS-232 port
Dimensions:	14.5 in. (37 cm) × 17 in. (43 cm) × 20 in. (51 cm) (*h*×*w*×*d*)
Weight:	53 lb. (24 kg) with temperature-controlled tray and column oven compartments
Power requirements:	100/120 VAC, 220/240VAC, 50/60 Hz, 450VA
Environmental:	10 to 40 °C; 5 to 95% relative humidity, noncondensing
Safety/EMC compliance:	CSA, TÜV, UL, FCC, CE Mark, EMC and Low Voltage Directives

Installation

This chapter describes the site requirements, installation procedures, and performance testing for the Thermo Scientific SpectraSYSTEM AS3000 autosampler.

Before you unpack the autosampler, read the instructions on lifting the autosampler (see "Lifting the Autosampler" on page 17).

The following items are factory installed: a 250 µL syringe, three sample trays, a sample needle, and a 100 µL sample loop.

Contents

Startup Checklist

This list summarizes the tasks that are required to install your autosampler. Complete installation information is contained in this chapter.

To install the autosampler, use these tools:

- Phillips screwdriver (1)
- 1/4 in. open-end wrench (2)
- Small, standard screwdriver (1)

Unpacking	
❒ Unpack and inspect your instrument.	"Unpacking the Autosampler" on page 18
❒ Remove cardboard/foam packing insert from the sample compartment (tub).	
❒ Check for parts shortages.	
❒ Read the Safety Information section.	
Back panel	
❒ Place the autosampler so that the back panel is accessible.	"Site Requirements" on page 16
❒ Install filter/solvent line and flush bottle.	
❒ Connect the power cord.	"Powering-Up the Autosampler" on page 29
❒ Connect the autosampler to the building ground.	"Connecting the Autosampler to the Building Ground" on page 19
❒ Hardwire the external events terminals and make the connections to other SpectraSYSTEM instruments.	
❒ For an autosampler under SpectraNET control, make the hardwire connections to other SpectraSYSTEM instruments and connect the instruments to the SN4000 system controller.	"Connecting the System Synchronization Harness" on page 37 and "Connecting the SpectraSYSTEM Devices to the SN4000 Controller" on page 31

Front panel	
❒ Install the solvent tray/waste container.	"Installing the Solvent Waste Container" on page 22
❒ Connect the autosampler to the pump: On instruments with the column oven installed, connect the pump outlet line to the solvent preheat tube.	"Connecting the Pump to the Autosampler" on page 24
❒ Mount the LC column: On instruments with the column oven installed, install the column between the column mounts, secure the column mounts to the oven, and connect the inlet end to injection valve port 5.	"Installing the Column" on page 25
❒ Connect the autosampler to the detector. (Connect 12 in., 0.010 in. ID tubing to the inlet side of flowcell.)	"Connecting the LC Column's Outlet to the Detector's Flowcell Inlet" on page 26
Startup/priming	
❒ Turn on the autosampler and check the initial response to power-on.	"Powering-Up the Autosampler" on page 29
❒ Using the 250 µL syringe, flush the lines with 500 µL of solvent.	
Performance verification	
❒ Run a standard sample (a test mix is shipped with the instrument).	"Performance Verification" on page 49
❒ Date and retain run results with instrument documentation.	
❒ Customize the READY participation settings.	"Ready Participation" on page 48
❒ Run an unretained sample to determine the system gradient delay time.	"Gradient Delay" on page 99
❒ Optimize the needle height. The default value of 2 mm is appropriate for most applications.	"Needle Height" on page 95
Installed by:	
(Name)	(Date)

Site Requirements

These topics describe the space and electrical requirements for the SpectraSYSTEM autosampler:

- "Space" on this page
- "Electrical" on page 17

Space

The autosampler weighs 24 kg (53 lbs) and requires a team effort to lift. Before you lift the autosampler, read the lifting instructions in "Lifting the Autosampler" on page 17. The instrument requires a bench space at least 14.5 in. (37 cm) × 17 in. (43 cm) × 20 in. (51 cm) (*h*×*w*×*d*). Be sure to allow adequate ventilation away from heating or air conditioning ducts. Avoid exposing the autosampler to direct sunlight. If used with the SpectraSYSTEM pump and detector, place the autosampler in the middle (see Figure 8).

Tip You might want to set your instrument on the benchtop so that you have easy access to the back panel.

Figure 8 does not show the vacuum membrane degasser. Place the vacuum membrane degasser to the left of the LC pump.

Figure 8. SpectraSYSTEM LC system benchtop setup

LC Pump Autosampler Detector

CAUTION If you use hazardous solvents, be sure to use the proper venting apparatus.

Electrical

Your autosampler automatically configures itself to operate from the local line voltage (100 to 260 VAC at 50 or 60 Hz). You do not need to set the line voltage. The instrument arrives with the necessary fuses installed.

To avoid intermittent failures and unexplained occurrences (lockup, loss of memory, incorrect operation) that are directly related to poor power, provide a stable, spike-free power source. Thermo Fisher Scientific recommends the use an uninterruptible power supply (UPS) for maximum protection from power-related failures.

CAUTION The UPS unit must meet the appropriate safety standards and be certified by authorized agencies for your country (Authorized agencies include TÜV, CSA, UL, and so on. The CE mark is accepted in most countries.) Thermo Fisher Scientific cannot guarantee that the LC system meets the appropriate safety standards when you connect uncertified devices to the autosampler.

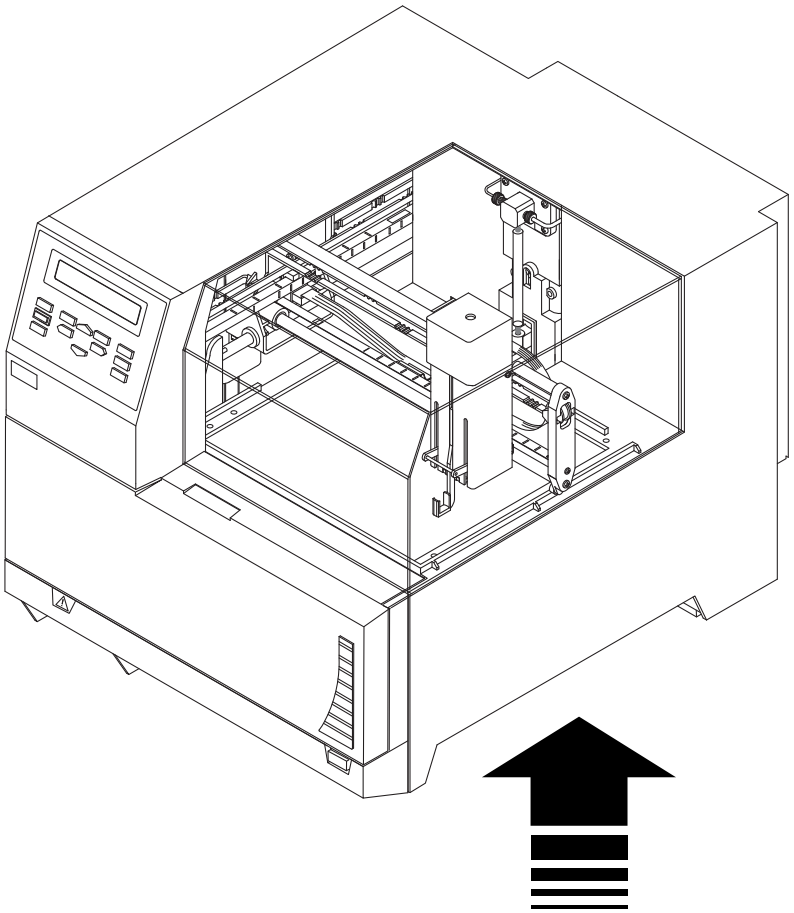
Lifting the Autosampler

The autosampler, at 24 kg (53 lbs) and a height, width, and depth of 37 × 43 × 51 cm (14.5 × 17 × 20 in.), is too heavy and too bulky for one person alone to lift safely. In addition, any jarring or uneven movement can cause misalignment of the autosampler's XYZ arm.

In accordance with international safety regulations, lifting the autosampler requires a team effort. Moving the autosampler from one benchtop to another requires two people to simultaneously lift the autosampler onto a movable cart. Using the lift points underneath the left and right sides of the autosampler, have one person lift the left side of the autosampler while the second person lifts the right side of the autosampler.

Figure 9 shows the lift point underneath the right side of the autosampler.

Figure 9. Lift point on the right side of the autosampler



Unpacking the Autosampler

Your autosampler arrives in a specially designed shipping container to protect it from damage during transit. When it arrives, inspect all containers and immediately report any damage in transit to the transportation company. Have the carrier note the shipping crate's condition on both the delivery receipt and the freight bill. The carrier is responsible for all damage incurred in shipment.

The SpectraSYSTEM autosampler arrives with the sample trays and syringe installed. A cardboard shipping insert on top of the sample trays restrains the XYZ arm and holds the sample trays in place during shipment. Remove the shipping insert. Retain both the shipping insert and shipping crate should future shipment become necessary.

After unpacking, inspect your autosampler and its accessories for missing parts, physical damage, or both. If you find damage, notify both the carrier and your Thermo Fisher Scientific representative. You must obtain authorization from Thermo Fisher Scientific prior to returning any goods.

The shipping kit contains the following:

- Autosampler
- Accessory Kit (See Chapter 8, “Replaceable Parts.”)
- Vial Kit (See Chapter 8, “Replaceable Parts.”)
- Declaration of Conformity
- SpectraSYSTEM documentation CD (contains the user guides for the SpectraSYSTEM family of LC products)

The accessory kit contains three sample trays and the vial kit contains a supply of vials, silicone and Teflon™ septa, and screw-top vial caps. You can order additional vials from Thermo Fisher Scientific. To ensure smooth autosampler operation, use standard size vials.

For information about installing the sample trays in the autosampler tray compartment (sample tub), see “Installing the Sample Trays” on page 27.

Connecting the Autosampler to the Building Ground

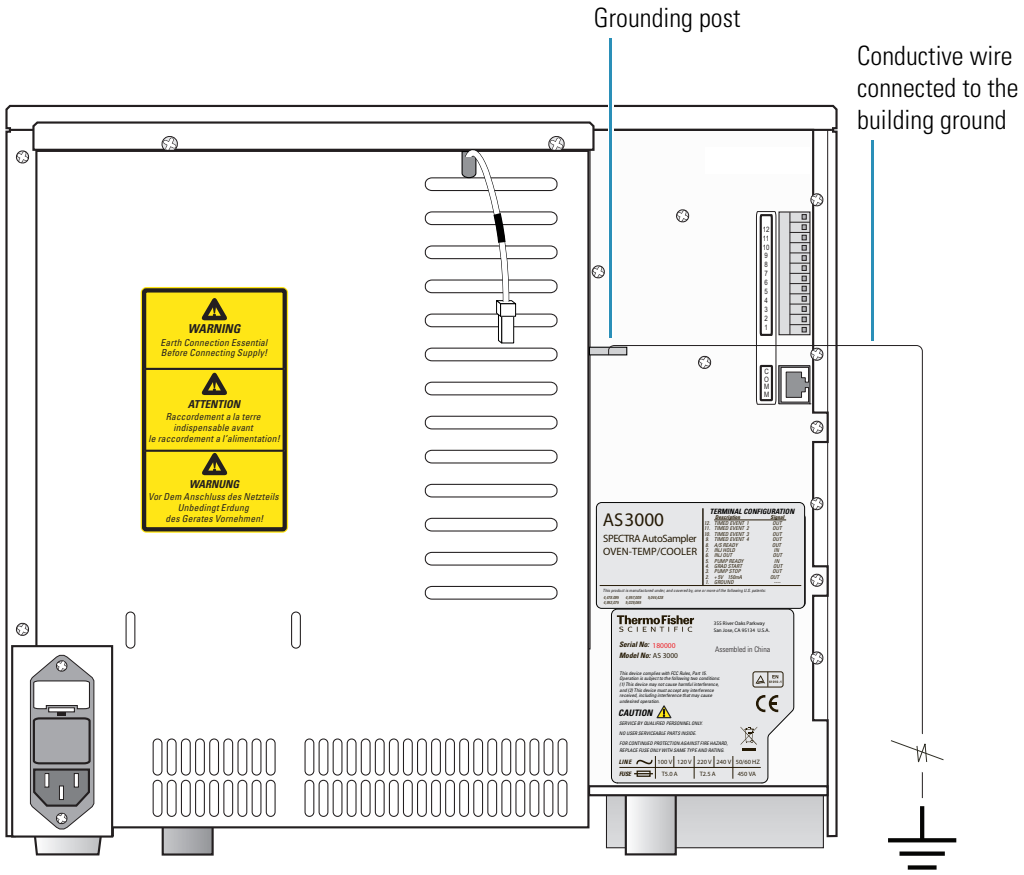
Before you turn on the autosampler, connect the grounding post on the back of the autosampler to the building ground.

CAUTION To avoid electric shock, make sure that the autosampler is connected to ground. Connect the power cord to a power outlet with a protective earth ground (see “Powering-Up the Autosampler” on page 29). Connect the grounding post on the back of the autosampler to the building ground.

❖ To connect the autosampler to the building ground

1. Connect one end of a conductive wire to the grounding post on the back of the autosampler (see Figure 10).
2. Connect the other end of the conductive wire to the building ground, for example, to an exposed metal fixture such as the building's water pipe.

Figure 10. Autosampler grounding post connected to ground



Connecting the Plumbing

To install the solvent bottles and connect the plumbing, follow these procedures:

Connecting the Flush Solvent Bottle

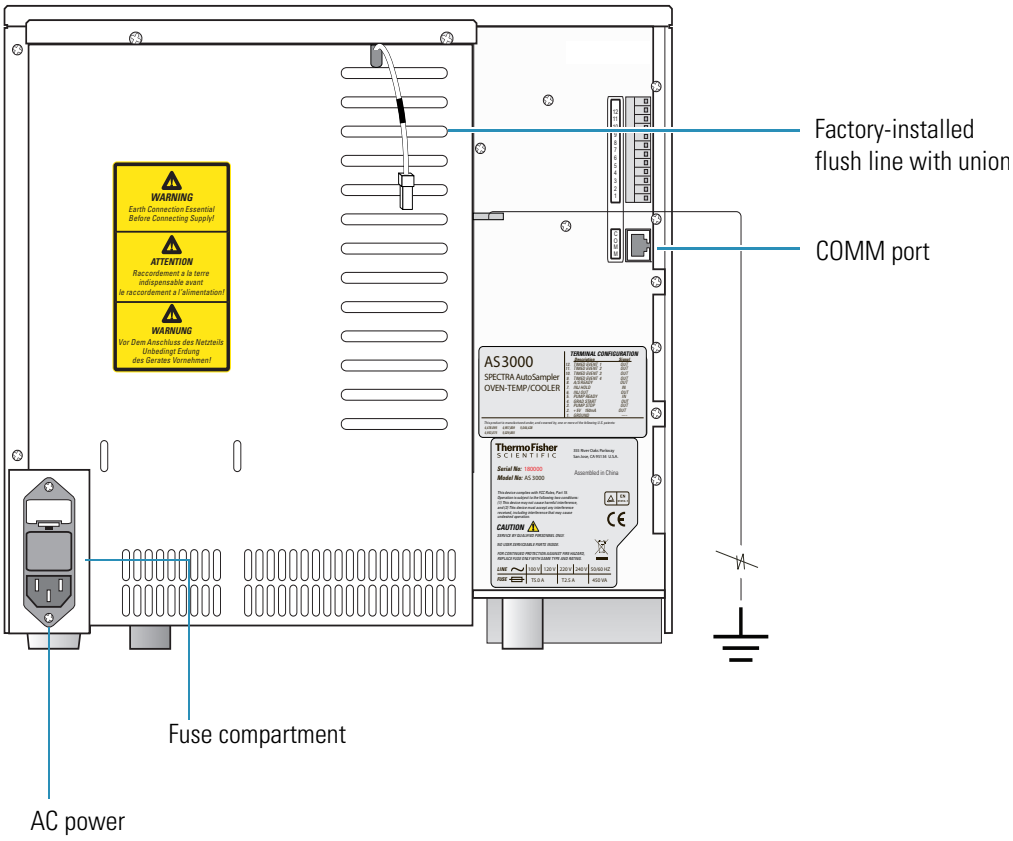
Your accessory kit includes a 250 µL flush-solvent bottle and cap. The cap assembly comes with the 1/8 in. OD × 1/16 in. ID Teflon inlet line and high-molecular-weight polyethylene filter attached.

Note If you want to use your own solvent bottle or another container, be sure that it is clean and chemically inert. For best results, use glass bottles.

❖ To install the flush bottle

1. Rinse the bottle with LC-grade solvent to remove any dust. Fill the bottle with the appropriate LC-grade solvent.
2. Insert the filter into the solvent bottle and screw on the cap. Make sure that the inlet filter rests on the bottom of the solvent bottle.
3. Connect the free end of the Teflon inlet line to the flush line on the back of the autosampler (see Figure 11).

Figure 11. Autosampler back panel connections



CAUTION Many LC solvents are hazardous. Make sure to handle them properly and dispose of any waste solvents in an environmentally correct manner.

4. Place the flush bottle in a secondary container.

Installing the Solvent Waste Container

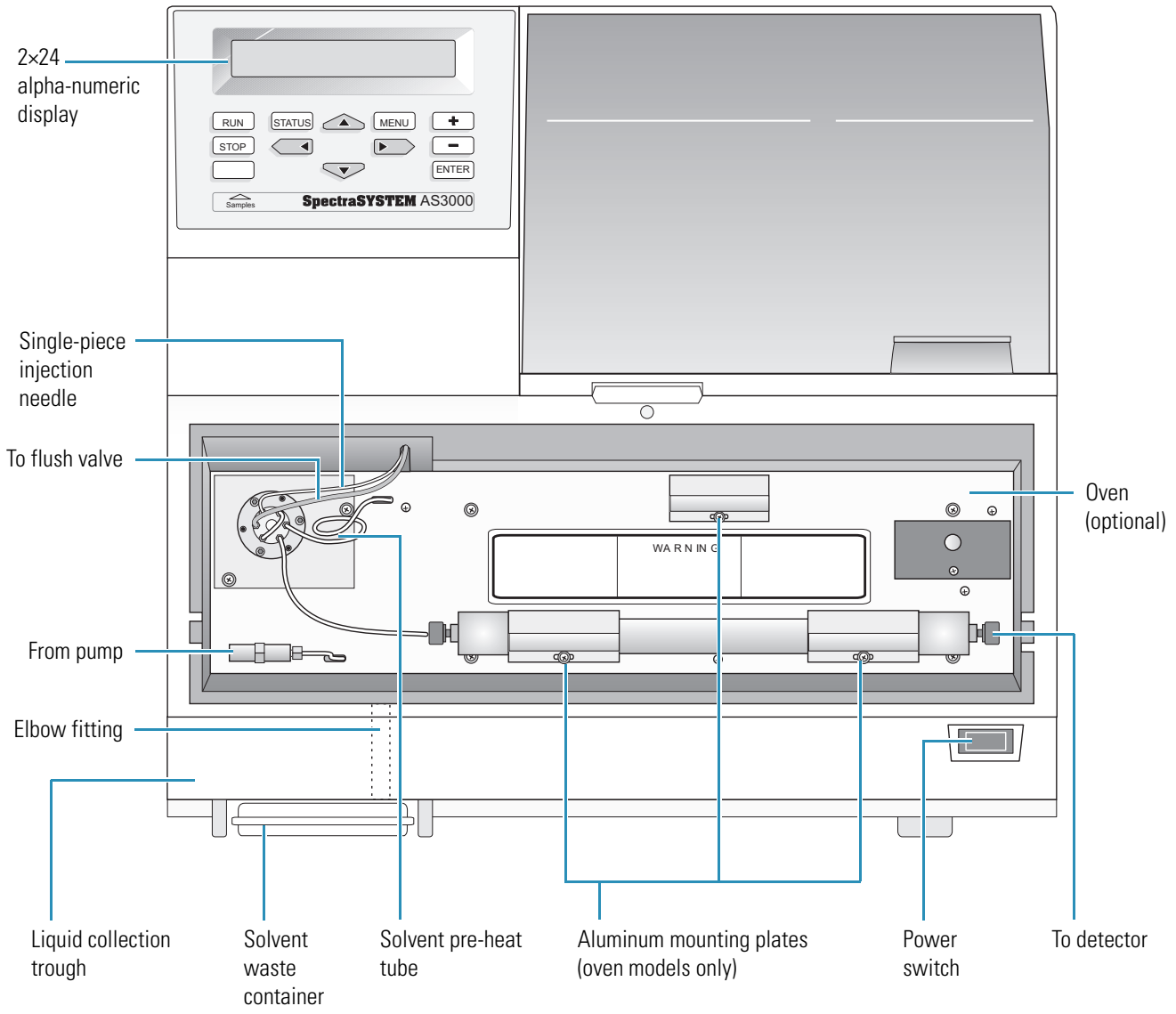
The solvent waste container (the flat, white plastic container included in your accessory kit) can hold 100 mL of waste. To install it, slide it into the compartment under the front left side of the instrument.

Note The general caution label on the left side of the autosampler below the column oven compartment is a reminder to empty the solvent waste container. The default flush volume for an injection cycle is 400 µL. This means that you can overfill the solvent waste container within 250 injections.

Note The back of the compartment prevents the tray from sliding too far under the instrument.

Orient the tray so that the black plastic, barbed elbow fitting is directed through the hole in the liquid collection trough (across the front of the instrument) as shown in Figure 12.

Figure 12. Autosampler front panel connections



Note Sometimes the barbed elbow fitting becomes dislodged during shipping. Be sure that the end of the barbed fitting is directed through the hole (not into the trough).

For larger injection applications, or for those requiring frequent flushing, you might want to install a piece of Tygon™ tubing onto the waste port to allow the waste to flow to a carboy or other large container for waste collection.

To hook up your waste container, locate the Tygon tubing included in your accessory kit and connect it to the end of the black, barbed fitting. To prevent backflushing into the lines, place the container lower than the instrument. Direct the Tygon tube into your container.

Connecting the Pump to the Autosampler

Note Your standard accessory kit contains two pieces of 0.010 in. ID stainless-steel tubing (one 4 in. piece and one 12 in. piece). The 12 in. piece is included to make the column-detector connection if the detector is not a SpectraSYSTEM detector. If you are connecting the pump to a SpectraSYSTEM detector, you can use the 12 inch tubing to make the pump-autosampler connection.

The autosampler arrives with the column oven assembly and the flush and needle lines already installed. The stainless-steel, low dead-volume union that connects the pump outlet tubing to the column oven tubing is also factory installed. You have to connect the pump to the zero dead-volume union (ZDV). The preheat line runs behind the column oven, through the oven's mantle, up through the service loop, and into the injection valve. For standard analytical configurations, this line holds approximately 180 µL of preheated solvent. Instruments with 0.007 in. narrow-bore preheat tubing provide approximately 22.7 µL of preheated solvent.

❖ To connect the pump to the preheat tube

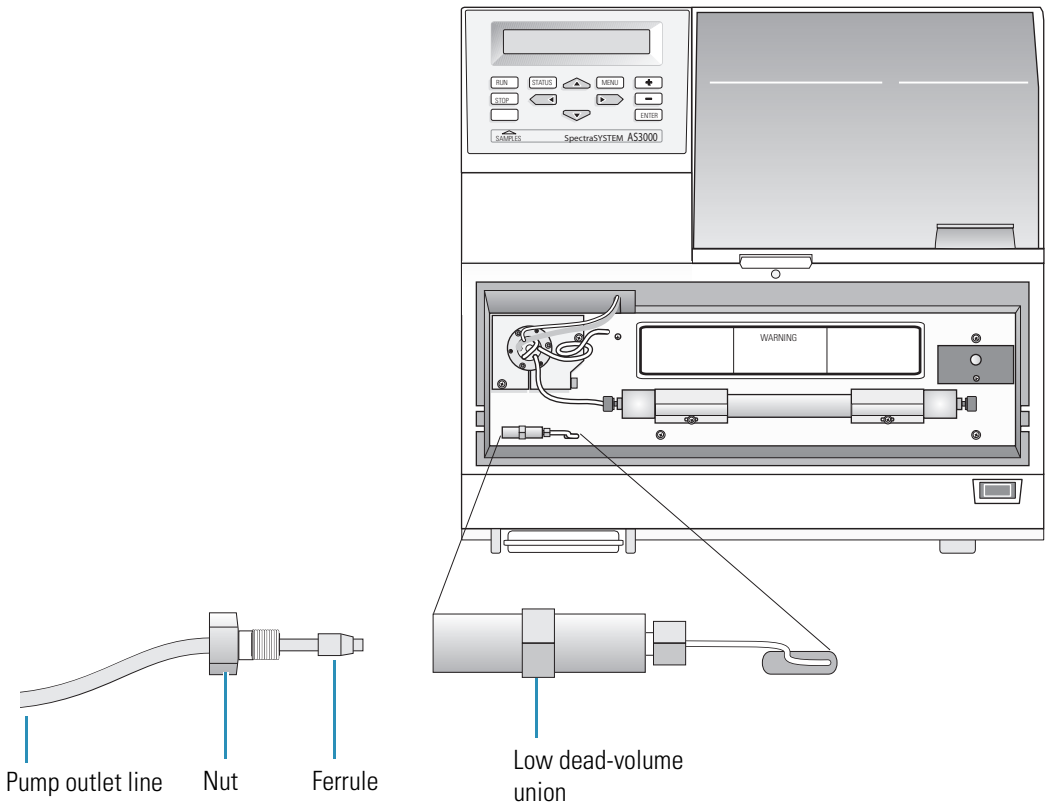
1. Remove the column oven cover (if you have not already done so) and set it aside.

   Tip The cover fits snugly. To remove it, place your fingers or thumb in the indentation on the top edge of the column door and firmly pull it back toward you.

2. Route your pump outlet line through the lower notch on the left side of the autosampler.
3. Connect the line to the low dead-volume union on the preheat tube as follows:
   a. Locate the ferrule included in your accessory kit.
   b. Thread the tubing through the fitting, and place the ferrule on the end of the preheat tube.
   c. Use two 1/4 inch wrenches to tighten the connection. Use one wrench to hold the union body and one to tighten the nut 1/8-turn past the stop.

Figure 13 shows the connection between the LC pump outlet and the preheat tube inlet.

Figure 13. LC pump outlet-to-preheat tube inlet connection



Installing the Column

The LC column is placed between two aluminum column mounts that attach to the column oven's plate. The aluminum mounts improve the heat transfer from oven to column.

The LC column secures directly onto the front of the oven plate. The instrument arrives with the column mounts already installed.

❖ To mount the LC column

Tip To ensure that no air gets into the column, remove air from the solvent lines before connecting the column.

1. Use a flat blade screwdriver to loosen the mounting screws and to remove and separate the column mounts.

Tip Thermo Scientific columns are usually connected so that liquids flow from left to right as you read the label. If you are not using a Thermo Scientific column, refer to your column documentation to orient it in the correct direction of flow.

2. Place the column between the column mounts, and replace the mounting screws to secure the column in place.

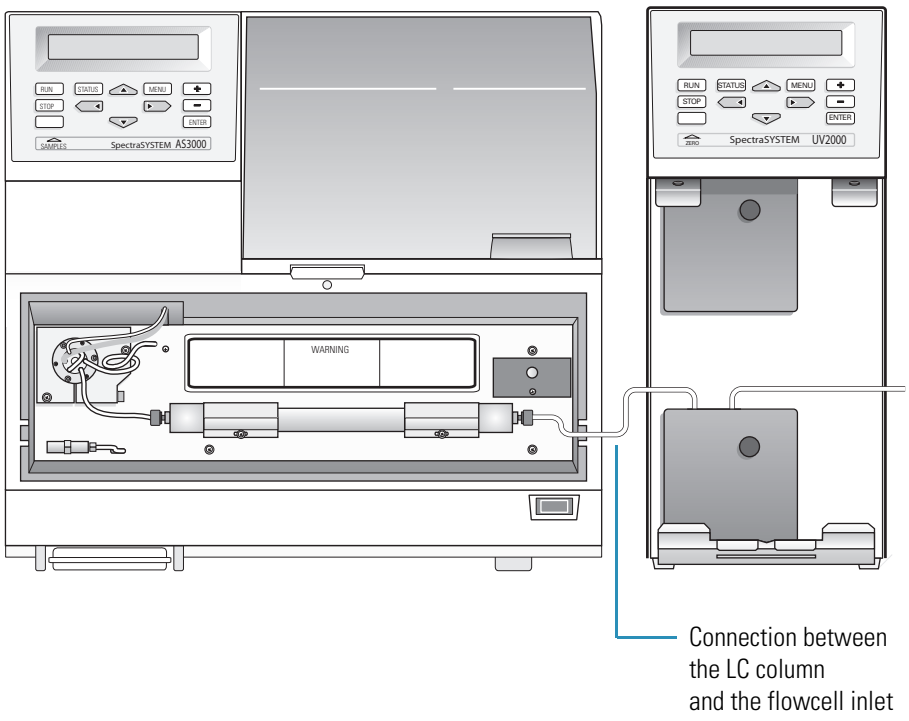
Connecting the LC Column's Outlet to the Detector's Flowcell Inlet

The accessory kit includes a 12 in. length of 0.010 in. ID tubing that you can use to connect the LC column outlet to a non-SpectraSYSTEM detector's flowcell inlet. The flowcell of a SpectraSYSTEM detector has an inlet line of sufficient length to reach the LC column outlet.

❖ To connect the LC column's outlet to the detector's flowcell inlet

1. Depending on the detector, do one the following:
   - For a SpectraSYSTEM detector, using the appropriate high-pressure fittings, connect the flowcell's inlet line to the outlet of the LC column (see Figure 14).
   - For a non-SpectraSYSTEM detector, using the appropriate high-pressure fittings, connect one end of the 12 in. length of 0.010 in. ID tubing (found in the accessory kit) to the column outlet and the other end to the flowcell inlet.
2. Replace the column oven cover, snapping it firmly into place (see "Installing the Column Compartment Cover" on page 27).

Figure 14. LC column outlet-to-flowcell inlet connection



Installing the Column Compartment Cover

❖ **To install the column compartment cover**

1. Insert the tabs on the bottom of the cover into the front lower edge of the autosampler.
2. Route the solvent lines through the slots on the sides of the column compartment.
3. Snap the top of the cover in place.

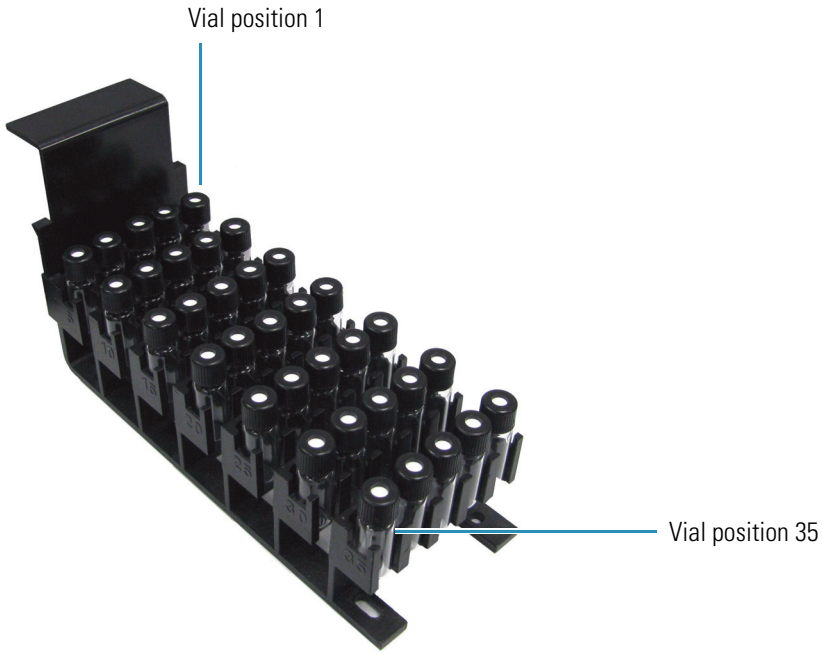
Installing the Sample Trays

Your autosampler comes with three sample trays and a supply of vials, silicone and Teflon septa, and screw-top vial caps. You can order additional vials from Thermo Fisher Scientific. To ensure smooth autosampler operation, use standard size vials.

IMPORTANT Be sure to use a 40 to 60 mil (1.0 to 1.5 mm) rubber or silicone septum with a 5 mil (0.1 mm) Teflon liner on one side.

Each tray contains 35 vials arranged in seven rows of five (see Figure 15).

Figure 15. 35-vial autosampler tray



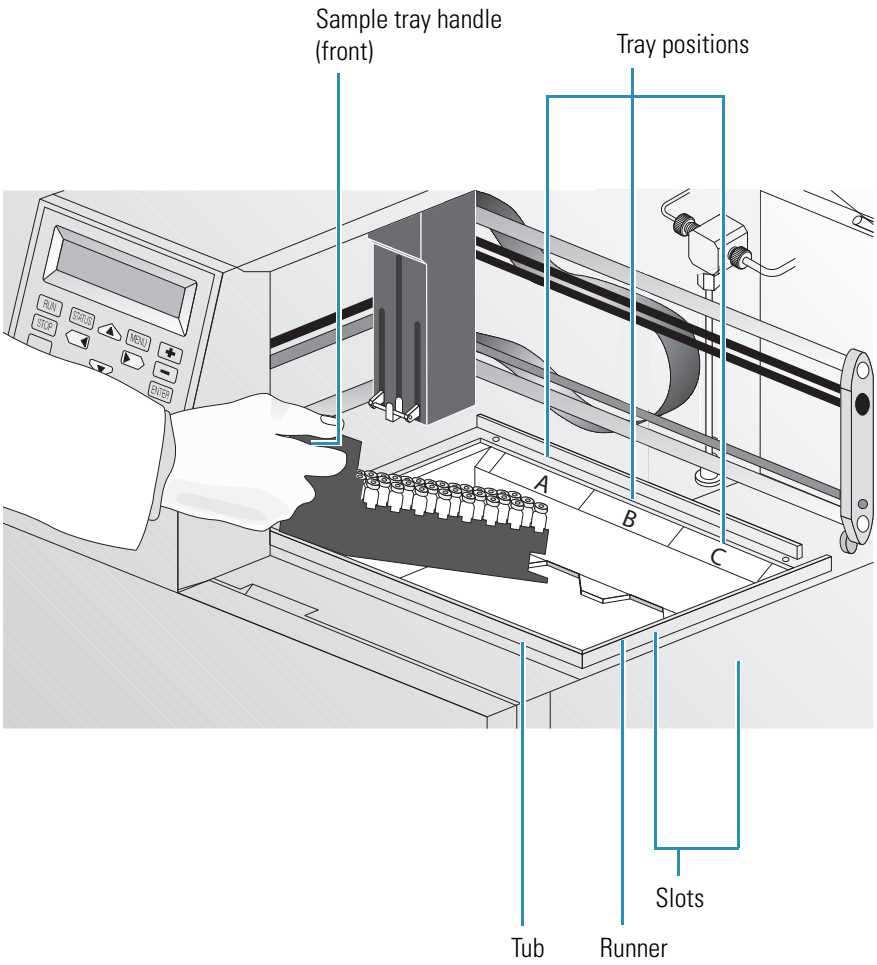
Trays A, B, and C are arranged in the autosampler from left to right. The first tray and vial position is A01; the last is C35.

IMPORTANT Be sure to use a 40 to 60 mil (1.0 to 1.5 mm) rubber or silicone septum with a 5 mil (0.1 mm) Teflon liner on one side.

❖ To install the sample trays

1. Hold the tray handle, tilting the back end down (see Figure 16).

   Figure 16. Installing a sample vial tray



2. Insert the tray's runners into the slots in position A at the back of the tub.
3. Lower the front of the tray into place and press down firmly to seat the tray.

> **Tip** Pull the handle back toward you until the tray snaps into place. Try moving each tray from side to side to be sure that it is seated completely.

4. Insert the other two sample trays into positions B and C.

> **IMPORTANT** Be sure that each tray is properly seated. Each tray's upper front edge should fit under the upper lip of the tub. If a tray is not completely seated, the autosampler does not correctly pick up or return vials to that sample tray.

Powering-Up the Autosampler

Your autosampler arrives with the correct fuses installed.

CAUTION Before you turn on the power switch, ensure that the autosampler is connected to the building ground (see "Connecting the Autosampler to the Building Ground" on page 19).

❖ **To connect the autosampler to line power and power-up the autosampler**

1. Locate the power cord in the shipping container (not in your accessory kit).
2. Insert one end into the AC power receptacle on the back panel and the other end to a grounded power outlet.
3. Turn on your instrument by pressing the on/off button on the lower right corner just below the front panel (see Figure 14 on page 26).

During startup, the instrument's self-diagnostic goes through the following sequence:

1. Completes a series of ROM checks.
2. Completes a series of RAM checks.
3. Activates the injection valve.
4. Verifies the proper connections on all installed options.
5. Verifies the proper operation of all sensors.
6. Homes (returns to starting position) all syringes and motors.

Priming the Autosampler

After you have completed the plumbing and power-up procedures and started your instrument, you must prime your autosampler to wet all of the lines. To do so, flush all syringes and lines with an appropriate solvent.

IMPORTANT The autosampler is shipped with methanol in the lines. Be sure to use a miscible solvent.

For complete instructions on using the autosampler keypad controls, see "Keypad Controls" on page 57.

❖ **To prime the autosampler**

1. If you have not already done so, fill your flush solvent bottle.
2. Press the MENU key and select **COMMANDS**.

   The Commands menu appears (see Figure 17).

   Figure 17. Commands menu

   ```
   Flush Sample Syringe
   Initialize Hardware
   Injector
   Arm
   ```

3. Select **Flush Sample Syringe** to access the menu shown in Figure 18.
4. Press the [+] and [–] keys to select **5000** µL. Then press the ENTER key to initiate the flush operation.

   Figure 18. Flush Sample Syringe menu

   ```
   Flush 5000 µL
   ```

The autosampler flushes the sample syringe and flush lines with the specified volume of flush solvent and returns the syringe to its starting position. The recommended 5000 µL flush takes approximately ten minutes. During the flush sequence, a confirmation message is displayed.

Communication: SpectraNet or Stand Alone

You can configure the SpectraSYSTEM autosampler to send information to other Thermo Scientific SpectraSYSTEM modules or to non-SpectraSYSTEM LC instruments, as follows:

- **SpectraNet mode**: Use the SpectraNet mode as a part of a SpectraSYSTEM chromatograph connected to other SpectraSYSTEM modules through the SN4000 system controller, and controlled by your chromatography data system.
- **Stand Alone mode**: Use the Stand Alone mode if you hardwired the autosampler to other non-SpectraSYSTEM HPLC modules through the remote input and output connectors, or connected it to older SpectraSYSTEM and non-SpectraSYSTEM instruments by means of specialized cables, communication protocols, or both.

This section contains the following topics:

For complete instructions on using the autosampler keypad controls, see "Keypad Controls" on page 57.

SpectraNet Mode

Configured as a SpectraSYSTEM module using SpectraNET communication and the SN4000 system controller, your autosampler is automatically controlled by your chromatography data system. The chromatography data system assumes almost all keyboard control and coordinates the majority of the autosampler's operations.

To set up the SpectraSYSTEM modules to communicate with the data system computer through the SN4000 controller, follow these procedures:

- "Connecting the SpectraSYSTEM Devices to the SN4000 Controller" on this page
- "Connecting the SN4000 Controller to the Data System Computer" on page 34
- "Setting the Configuration to the SpectraNet Mode" on page 35

To synchronize the input and output signals during a run, follow these procedures:

- "Setting Up the Autosampler and Pump Contact Closure Signals" on page 36
- "Connecting the System Synchronization Harness" on page 37

Connecting the SpectraSYSTEM Devices to the SN4000 Controller

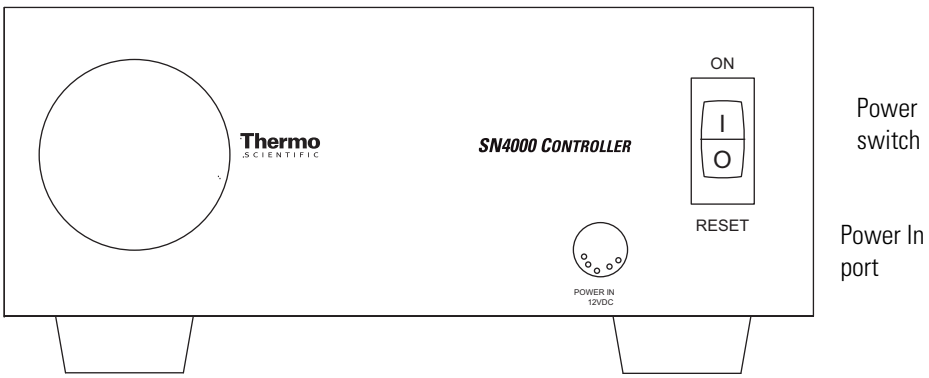
Use RJ11 cables to connect a SpectraSYSTEM UV/VIS or fluorescence detector, a SpectraSYSTEM pump, and a SpectraSYSTEM autosampler to the SN4000 controller. Use an RJ45 cable and an RJ45 to DB9 adapter to connect the SN4000 controller to the data system computer.

❖ To connect the communication cables to the SN4000 controller

1. Turn the SN4000 power switch to the Off position, and then disconnect the 12 V dc power supply

   Figure 19 shows the power switch and the Power In port on the back panel of the SN4000 controller.

   Figure 19. SN4000 controller back panel

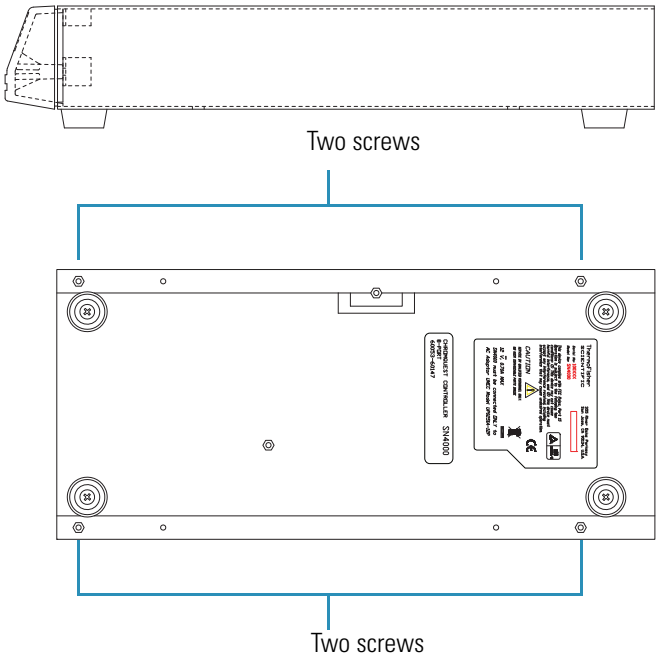


CAUTION Before you remove the top cover of the SN4000 controller, turn the power switch on the back of the controller to the Off position, and then disconnect the 12 V dc power supply.

2. Remove the top cover of the SN4000 controller as follows:

   a. Turn the SN4000 controller upside down to access the four screws that secure the top cover to the case (see Figure 20).

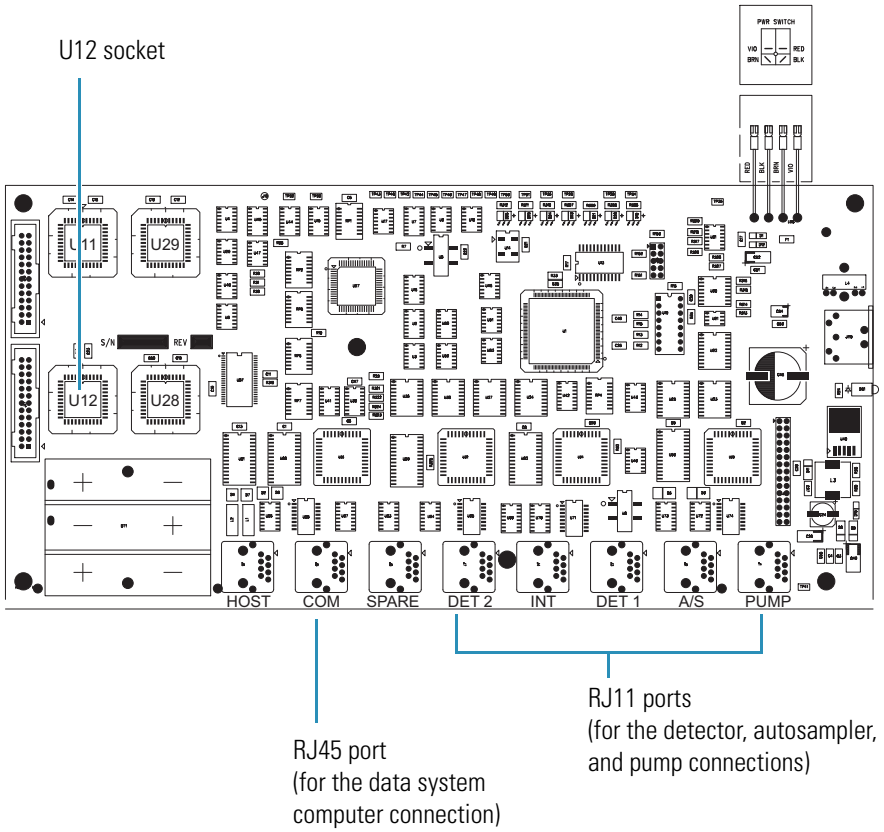
   Figure 20. Screws that secure the top cover to the case (bottom view of the controller)



   b. Using a #2 Phillips head screwdriver, remove the screws and slide the top cover off the case.

   Figure 21 shows the SN4000 PCB.

Figure 21. SN4000 PCB



3. Verify that the EPROM firmware version 9.05 (P/N A4636-129) is installed in socket U12 on the SN4000 PCB and that sockets U11, U28, and U29 are empty.
4. To connect a SpectraSYSTEM pump to the SN4000 controller, do the following:
   a. Connect one end of an RJ11 6-pin, 4-wire cable to the PUMP port on the SN4000 PCB.
   b. Route the cable through the access hole on the back panel of the controller.
   c. Connect the other end of the cable to the COMM port on the back panel of the pump.
5. To connect a SpectraSYSTEM autosampler to the SN4000 controller, do the following:
   a. Connect one end of an RJ11 6-pin, 4-wire cable to the A/S port on the SN4000 PCB.
   b. Route the cable through the access hole on the back panel of the controller.
   c. Connect the other end of the cable to the COMM port on the back panel of the autosampler.

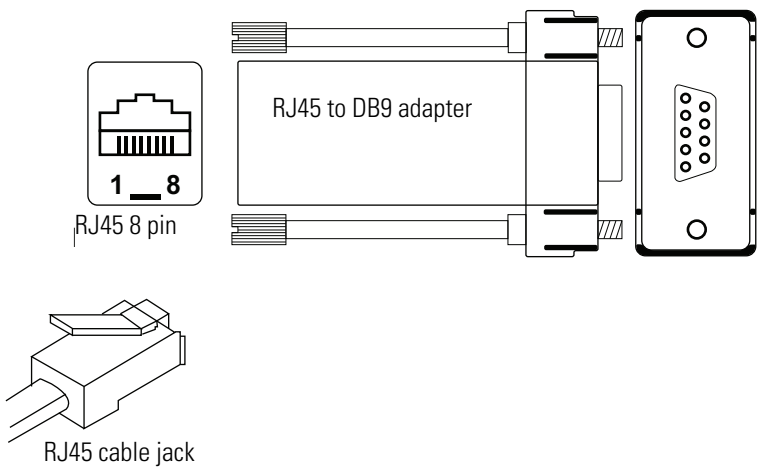
6. To connect a SpectraSYSTEM UV/Vis or fluorescence detector to the controller, do the following:
   a. Connect one end of an RJ11 6-pin, 4-wire cable to the DET 1 port on the SN4000 PCB.
   b. Route the cable through the access hole on the back panel of the SN4000 controller.
   c. Connect the other end of the cable to the COMM port on the back panel of the UV/Vis or fluorescence detector.

Connecting the SN4000 Controller to the Data System Computer

❖ **To connect the SN4000 controller to the data system computer**

1. Connect one end of an RJ45 8-pin, 8-wire cable to the Com port on the SN4000 PCB.
2. Route the cable through the access hole on the back panel of the SN4000 controller.
3. Connect an RJ45 to DB9 adapter to the other end of the cable (see Figure 22).

Figure 22. RJ45 to DB9 adapter



4. Connect the DB9 adapter to one of the RS-232 ports on the back panel of the data system computer.
5. Reinstall the SN4000 controller top cover. Using a #2 Phillips screwdriver, tighten the screws that secure the cover to the case.
6. Reconnect the power cable from the 12 V dc power supply to the Power In inlet on the back panel of the SN4000 controller.

Setting the Configuration to the SpectraNet Mode

To control the SpectraSYSTEM LC system from a data system computer, the SpectraSYSTEM autosampler must be set to the SpectraNet communication mode.

❖ To configure your autosampler to use SpectraNet communication

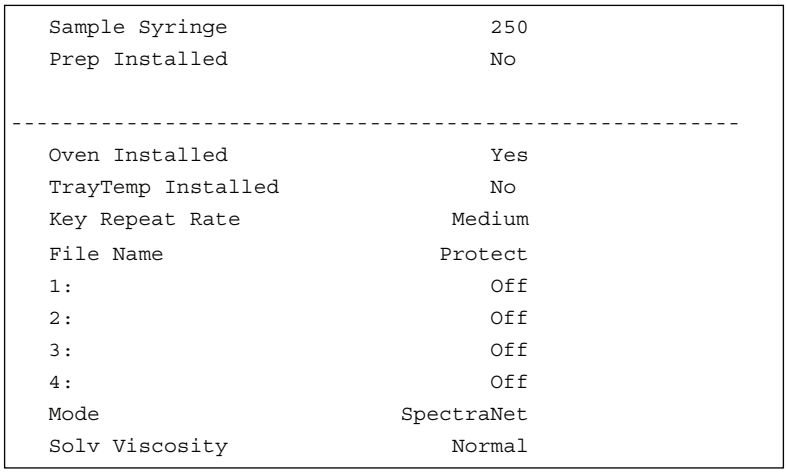
1. Turn on your autosampler, wait for the power-up sequence to complete, and then press the MENU key.

   The main menu appears (see Figure 39 on page 61).

2. Select **OPTIONS > Configurations**.

   The Configurations menu appears (see Figure 23).

   Figure 23. Configurations menu in SpectraNet mode



3. Move the cursor to the Mode field, and press the [+] and [–] keys to select **SpectraNET**. Then press ENTER to accept the field value and exit the Configurations menu.

Setting Up the Autosampler and Pump Contact Closure Signals

Before you can control the run timing of the SpectraSYSTEM modules from a data system computer, you must configure the polarity of the contact closure signals for both the autosampler and the pump.

❖ To set up the autosampler's contact closure signals

1. On the autosampler keypad, press the MENU key.

   The main menu appears (see Figure 39 on page 61).

2. Press the LEFT ARROW (◀) or RIGHT ARROW (▶) key to select **OPTIONS**, and then press the ENTER key.

3. Press the DOWN ARROW (▼) key to select **Input Polarity**, and then press the ENTER key.

   The Input Polarity screen appears with Pump Ready Active selected (see Figure 24).

   Figure 24. Input Polarity screen

   ```
   Pump Ready Active              Hi
   Inj Hold Active                Lo
   ```

4. Use the [+] and the [–] keys to select **Hi**.

5. Press ENTER to accept the field value and exit the Configurations menu.

❖ To set up the pump's contact closure signals

1. On the pump keypad, press the MENU key.

2. Press the LEFT ARROW (◀) or RIGHT ARROW (▶) key to select **OPTIONS**, and then press the ENTER key.

3. Press the LEFT ARROW (◀) or RIGHT ARROW (▶) key to select **More**, and then press the ENTER key.

   The More menu for the pump appears.

   Figure 25. More menu for the SpectraSYSTEM pump

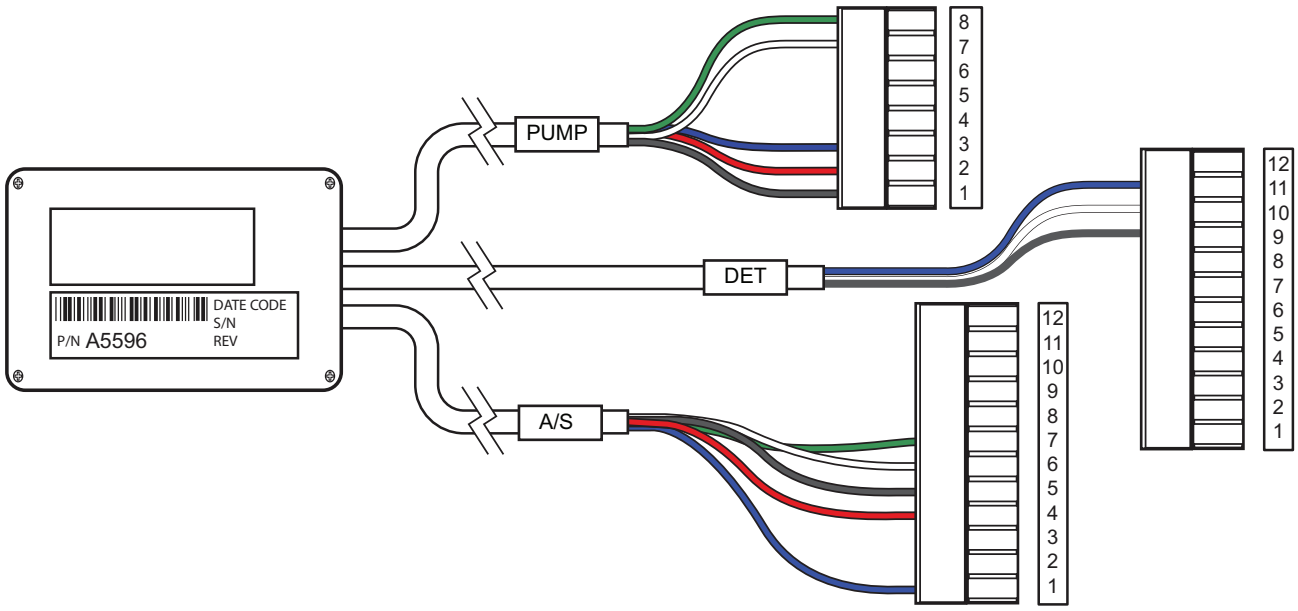
   ```
   Pressure Units                      PSI
   Purge Mode              Flow
   — — — — — — — — — — — — — — — — — — —
   Delay Volume                        0.0
   Cursor Speed                     Medium
   — — — — — — — — — — — — — — — — — — —
   Status Lock                         Off
   Ready Output Active                  Hi
   ```

4. Press the DOWN ARROW (▼) key to select **Ready Output Active**, and then press the ENTER key.
5. Press the [+] and the [–] keys to select **Hi**.
6. Press the ENTER key to accept the value and exit the More menu.

Connecting the System Synchronization Harness

The timing of the SpectraSYSTEM modules is synchronized through a contact closure wiring harness (see Figure 26). The harness has one connector each for the pump, detector, and autosampler.

Figure 26. Contact closure wiring harness for the SpectraSYSTEM LC system

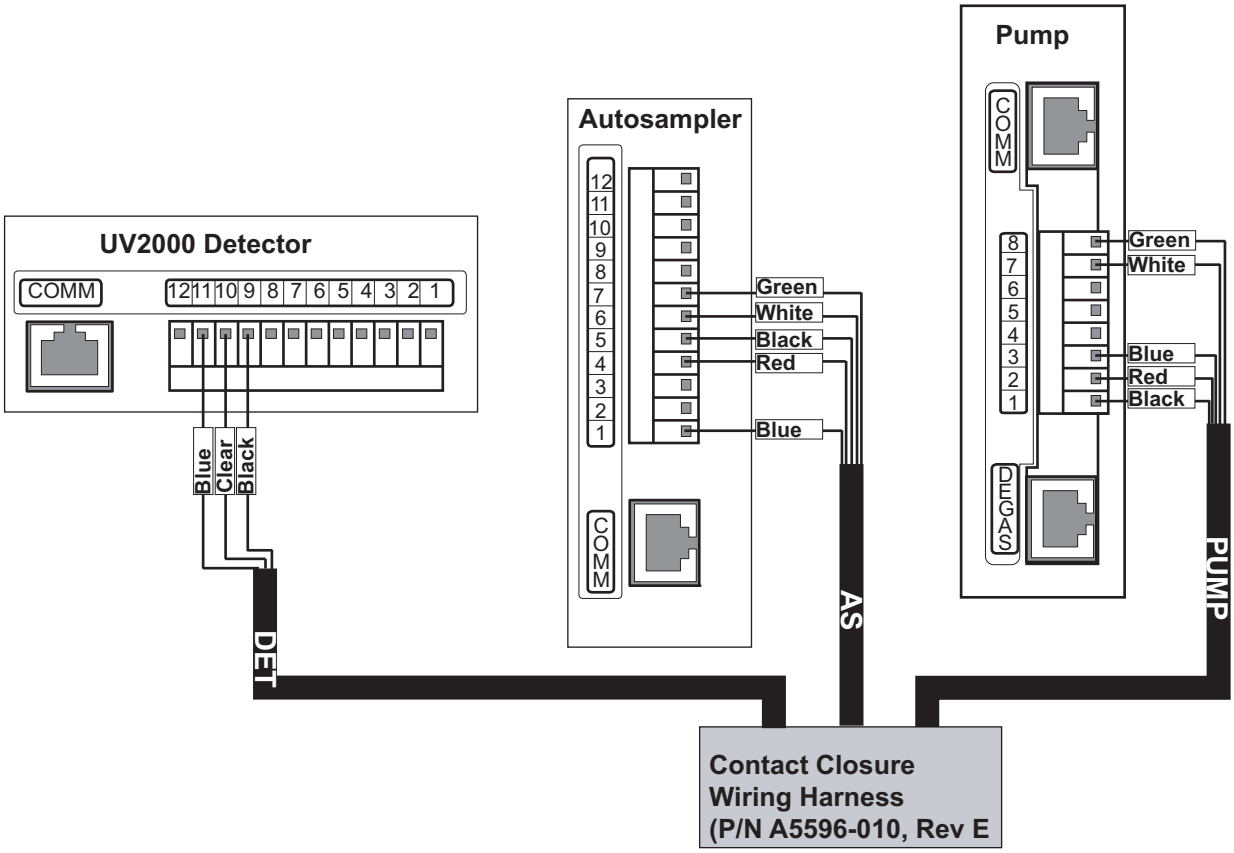


❖ To connect the system synchronization harness

1. Connect the cable labeled PUMP to the 8-pin terminal on the back panel of the SpectraSYSTEM pump (see Figure 27).
2. Connect the cable labeled AS to the 12-pin terminal on the back panel of the SpectraSYSTEM autosampler (see Figure 27).
3. Connect the cable labeled DET to the 12=pin terminal on the back panel of the detector (see Figure 27).

Note For information on connecting the SpectraSYSTEM UV8000 Vacuum Degasser and PDA Detector module to the LC pump and autosampler, refer to the *SpectraSYSTEM UV8000 Vacuum Degasser and PDA Detector Hardware Manual*.

Figure 27. Contact closure connections for a typical SpectraSYSTEM LC system

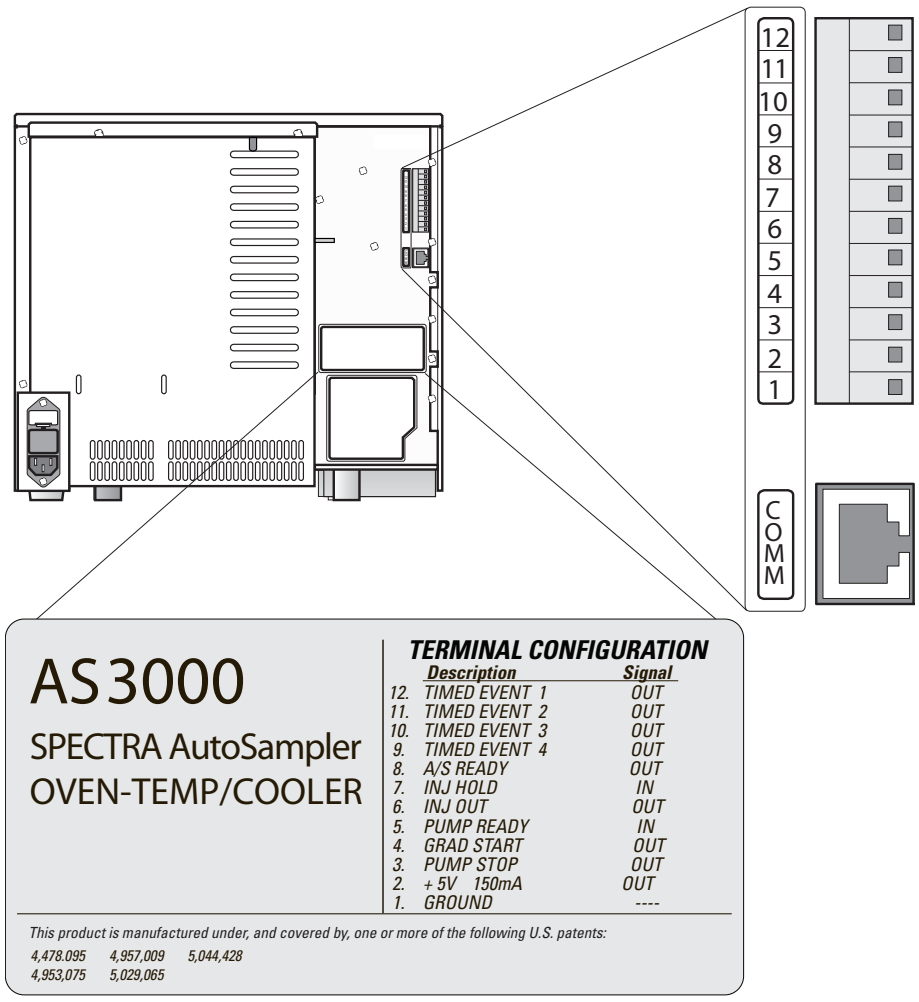


Stand Alone Mode

Use the Stand Alone mode to operate SpectraSYSTEM modules without data system control.

With the 12-pin terminal on the instrument's back panel, you can hardwire the injection synchronization signals between your autosampler and other components in an LC system (see Figure 28).

Figure 28. 12-pin terminal



Use a combicon connector (found in the accessory kit) to attach control wires from other LC devices to the 12-pin terminal. Once you have made all the connections, you can make and break connections without disturbing individual wires.

❖ To connect a wire to the combicon connector

1. Loosen the appropriate screw.
2. Insert the wire.
3. Tighten the screw.

With hardwire communications, the autosampler can start and stop a pump or detector, begin a gradient program (gradient pumps only), or control other features of the externally connected instruments. In addition, the terminal has four timed-function outputs that you can use to control valve-switching devices or other devices on a timed basis. To set up a timed-events program, see "Timed Events Menu" on page 72.

To set up your stand-alone system, follow these topics as appropriate:

Input and Output Terminal Specifications

The twelve terminals on the autosampler's back panel can be divided into two groups: outputs and inputs.

The output terminals, including Time Functions 1 through 4 (TF1-4), are open-collector outputs capable of sinking (handling) currents up to 600 mA at voltages up to 24 VDC. The active state is user programmable in the Timed Events menu (see "Timed Events Menu" on page 72).

The input terminals accept TTL-level inputs, with a transition activating each. The two inputs are tied to +5 VDC through pull-up resistors. A "Lo" level is from 0 to 0.8 V; a "Hi" level is from 2 to 5.5 V.

IMPORTANT All inputs and outputs are referenced to ground.

Pin Assignments

Table 1 list the input and output pin assignments.

Table 1. Input and output pin assignments (Sheet 1 of 2)

Pin #	Assignment
Pin 1 GROUND:	Use this pin as the ground reference used for all inputs and outputs.
Pin 2 +5VDC:	Use this regulated +5 VDC supply to power pull-up resistors or external relays. The current is limited to a maximum of 300 mA to protect against external short circuits affecting the autosampler's operation.
Pin 3 PUMP STOP (output):	Use this pin to send a pulsed output signal to stop the pump after the last sample has been processed. It is activated when the last sample in the queue has completed its last injection, or when the shutdown file has been activated (using the special shutdown command). For more information on the Shutdown command, see "Automated Shutdown in the Stand Alone Mode" on page 123.
Pin 4 GRADIENT START (output):	Use this pin to send a signal to the pump to start its gradient program. The autosampler send this signal at a specified amount of time before it activates the Inject Out pin. The time is specified as the Gradient Delay value in the More menu (under Files). This momentarily active, preinjection signal permits the gradient to start prior to sample injection to compensate for the system delay volume. The result is an injection that occurs just as the gradient front reaches the injection valve. For more information on the gradient delay function, see "Gradient Delay."
Pin 5 PUMP READY (input):	Through this input, the pump signals the autosampler that the mobile-phase flow rate is stable and that the pump is ready for an analysis to begin. (You can connect other devices here as well.) This input must be continuously active for the autosampler to proceed with injections. From the Input Polarity menu, you can select whether a high or low level on this line means "ready." The default is Hi.
Pin 6 INJECT OUT (output):	Use this output pin to synchronize the timing of external devices (pumps, detectors, integrators, and so on) to the autosampler injection. This line sends a momentary signal each time the autosampler makes an injection. For the specifications of this output, see "Input and Output Terminal Specifications" on page 40.
Pin 7 INJECT HOLD (input):	Use this input pin to keep the autosampler in the hold state with sample in the loop. Releasing the hold causes an immediate injection. The signal must remain continuously active to hold injections. That is, when active, this signal delays an imminent injection until this line goes to the inactive state. You can use this function with external equipment to precisely control the timing of the injection valve activation. You can select the active level (Hi or Lo) of this input in the Input Polarity menu.

Table 1. Input and output pin assignments (Sheet 2 of 2)

Pin #	Assignment
Pin 8 A/S READY (output):	Use this pin to signal to other devices that the autosampler is in a READY condition.
Pins 9 thru 12 TF1-TF4 (timed outputs):	Use these user-programmed outputs to trigger column-switching valves or other external hardware. The time functions defined in the currently active file control these outputs (see "Timed Events Menu" on page 72).

Recommended Hardwire Connections for a Stand-Alone System

The recommended setup for a stand-alone LC system requires a pump, autosampler, UV/VIS or fluorescence detector, and an integrator. For best results, connect the following signals:

- Pump Ready
- Inject Hold (detector ready)
- Detector Run/Start
- Integrator Run/Start

To wire the appropriate pin connections, see Table 2. Pin connections for the SpectraSYSTEM modules are indicated in parentheses.

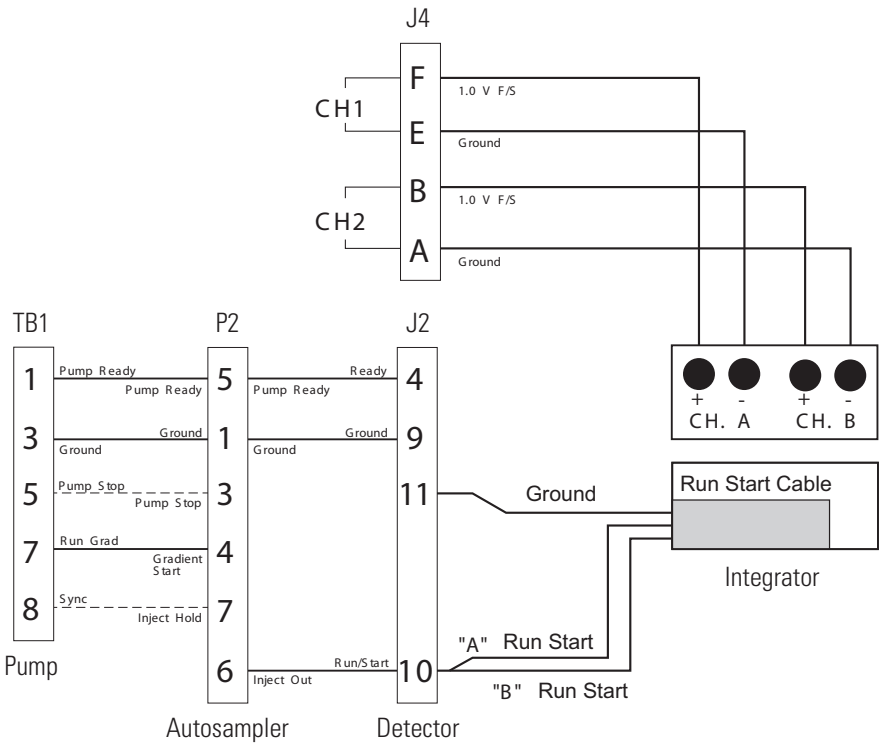
Table 2. Pin connections for a stand-alone system

Autosampler (pin connection)	Pump	Detector	Integrator
Ground (1)	Ground (3)	Ground (9) (11)	Ground (B1)
Pump Stop (3) (pulsed output)	Pump Stop (5)	N/A	N/A
Pump Ready (5) (input)	Pump Ready (1)	READY (4)	N/A
Inject Out (6)	N/A	RUN/START (10)	RUN/START "A" (B5) RUN/START "B" (B14)
Inject hold (7)*	Sync (8)	N/A	N/A
Gradient Start (4)	Run Grad (7)	N/A	N/A

* Indicates optional connections

Figure 29 illustrates the hardwire connections for the SpectraSYSTEM modules.

Figure 29. Hardwire connections for the SpectraSYSTEM modules



Communications Menu Parameters

For normal stand-alone operation, you do not need to change any of the values in the Communications menu. Keep the communications parameters set to the default values (see Figure 30). The following details for changing the polarity are provided for reference only.

Figure 30. Communications menu with the default values for stand-alone operation

```
Baud Rate       9600
Parity          NONE
Flow Control    NONE
Data Bits       8
Stop Bits       1
Echo            On
```

These are the allowable entries and default values for the Communications parameters.

Parameters	Allowable entries	Default values
Baud Rate	1200, 2400, 4800, or 9600 Bd	9600 Bd
Parity	None, Even, or Odd	None
Flow Control	None or XON/OFF	None
Data Bits	8 or 7	8
Stop Bits	1 or 2	1
Echo	On or Off	On

❖ To access the Communications menu

From the OPTIONS menu, select **Communications**.

Tip If the Communications menu does not appear as one of the OPTIONS menu choices, return to **OPTIONS > Configurations**, and verify that Mode is set to Stand Alone. The Communications field does not appear in the OPTIONS menu if Mode is set to SpectraNET.

Setting the Polarity of the Autosampler Signals

From the OPTIONS menu you can change the polarity of your autosampler's input and output signals as required by your other LC system modules. For example, when shipped, the Autosampler Ready Output is set to send a positive voltage (Hi) when the instrument is ready. If you change the Autosampler Ready Output Polarity to Lo, the instrument sends a 0-volt signal (Lo) when it is ready.

The following procedures explain how to change the polarity of the input and output signals.

❖ To change the polarity of an input signal

1. From the OPTIONS menu, select **Input Polarity**.
2. Press the [+] and [–] keys to select either **Hi** or **Lo** for the desired input signal shown in Figure 31.

Figure 31. Input Polarity menu

```
Pump Ready Active      Hi
Inj Hold Active        Lo
```

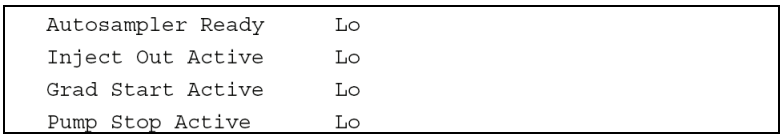
This menu shows the level for the active state for each input signal.

Tip To determine the current state of a particular signal, return to the main menu, and select **TESTS > Display Input States**.

❖ To change the polarity of an output signal

From the OPTIONS menu, select **Output Polarity**. Press the [+] and [–] keys to select either **Hi** or **Lo** for the desired output signal shown in Figure 32.

Figure 32. Output Polarity menu



This menu shows the level for the active state for each output signal.

Tip To determine the current state of a particular signal, return to the main menu, and select **TESTS > Display Output States**.

Table 3 and Table 4 show the output signal levels relative to the polarity settings and instrument status for the autosampler's input and output signals, respectively.

Table 3. Input signal levels

Signal	State	Polarity	
		Hi	Lo
Pump Ready	Ready	Hi*	Lo
	Not Ready	Lo	Hi
Inj Hold	Active	Hi	Lo*
	Inactive	Lo	Hi

* Default settings

Table 4. Output signal levels

Signal	State	Polarity	
		Hi	Lo
Autosampler Ready	Ready	Hi	Lo*
	Not Ready	Lo	Hi
Inject Out	Active	Hi	Lo
	Inactive	Lo	Hi*
Gradient Start	Active	Hi	Lo
	Inactive	Lo	Hi*
Pump Stop	Active	Hi	Lo
	Inactive	Lo	Hi*

* Default settings

Stand-Alone Communication to Integrators

If you are connecting the SpectraSYSTEM LC to an integrator, two specialized communication setups are available:

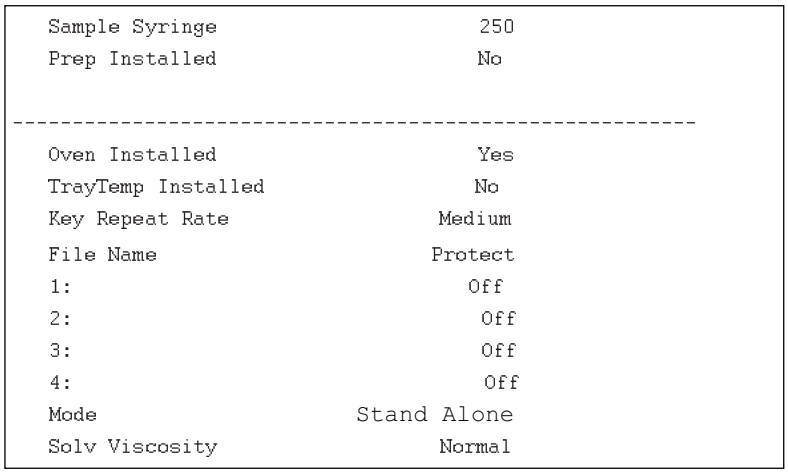
- Through an autosampler-to-integrator cable to ChromJet or DataJet integrators
- Through a binary-coded decimal (BCD) interface to older Thermo Scientific integrators or to non-Thermo Scientific Integrators

Before using either of these communications setups, you must configure your autosampler as a stand-alone module.

❖ To configure your autosampler as a stand-alone module

1. Turn on your autosampler, wait for the power-up sequence to complete, and then press the MENU key. Select **OPTIONS > Configurations** to access the Configurations menu (see Figure 33).

   Figure 33. Configurations menu with the Mode field set to Stand Alone



2. Select the **Mode** field, and press the [+] and [–] keys to select **Stand Alone**. Then press the ENTER key to accept the field value and exit the Configurations menu.

Your autosampler is now configured as a stand-alone LC module.

ChromJet or DataJet Communication

Using an autosampler-to-integrator cable available from Thermo Fisher Scientific, you can set up your autosampler to send vial information to a Thermo Scientific ChromJet (SP4400) or a DataJet (SP4600) integrator. For this connection you must have an RJ-45-to-RJ-45 cable and an integrator adapter (P/N A3981-010).

❖ To connect an integrator to the autosampler

1. Turn off your integrator.
2. Insert the RJ-45 connector (telephone connector) into the COMM port (telephone jack) on the back panel of the autosampler.
3. Connect the adapter to the other end of the cable. It orients in only one direction.
4. Plug the adapter-cable end into your DataJet or ChromJet integrator.
5. Turn on your integrator and type the following:
   - BAUD 9600
   - POKE#C12D,#FF
6. Hold down the CTRL key and press CALIB.

IMPORTANT You must reenter this sequence after any integrator soft reset. For more information, refer to the manual supplied with the integrator.

This sequence provides you with the four-line report from your autosampler shown in Figure 34.

Figure 34. Vial location and injection report

```
Sample Vial: A02 Inj Volume :        5.0   Oven Temp :   26
Inject Vial: A01 Inj/Vial   :        1/1   Tray Temp:     0
Vial Type  : SAMPLE   Cycle Time    0.8   Heater Temp:  24
File 1:  FILENAME     Set Number :      1
```

BCD Communication

The BCD communication option provides BCD communication from the SpectraSYSTEM AS3000 autosampler to older Thermo Scientific and non-Thermo Scientific integrators and computers. This option provides tray- or vial-location information (or both) to devices requiring either binary or binary-coded decimal data. If ordered after initial instrument delivery, the BCD assembly must be installed by a Thermo Fisher Scientific service representative. Installation instructions are provided in the BCD Option Kit. Contact your representative for further information.

Ready Participation

Because Enable is the factory setting for all of the selections on the Ready Participation menu, the Status screen indicates that the autosampler is ready for operation when these conditions are satisfied:

- The door is closed.
- The column oven is at the set temperature.
- The temperature controller for the tray compartment is at the set temperature.

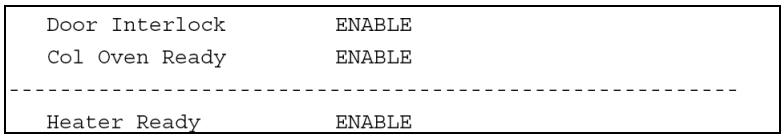
Customize the settings on the Ready Participation menu to meet your needs.

❖ To change the settings on the Ready Participation menu

1. From the main menu, select **OPTIONS > Ready Participation**.

   The Ready Participation menu appears (see Figure 35).

   Figure 35. Ready Participation menu



2. To change any of the above requirements, select the appropriate line, and press the [+] and [–] keys to change ENABLE to DISABLE.

Hardware Configurations

Your AS3000 autosampler is shipped with a 100 µL sample loop and a 250 µL sample syringe. You can change the syringe sizes from the Configurations menu (see Figure 36). This menu also reflects your other installed options (for example, the oven temperature control). If you want to adjust the rate at which fields cycle through the available choices, you can change the Key Repeat Rate entry. "Protecting Files" on page 91 discusses the File Protect function also available from this menu.

Figure 36. Configurations menu

```
   Sample Syringe                    250
   Prep Installed                    No

-----------------------------------------------------------
   Oven Installed                    Yes
   TrayTemp Installed                No
   Key Repeat Rate                Medium
   File Name                     Protect
   1:                                Off
   2:                                Off
   3:                                Off
   4:                                Off
   Mode                       SpectraNet
   Solv Viscosity                 Normal
```

Performance Verification

After you install your SpectraSYSTEM autosampler, run a standard sample, such as a 0.5 percent mixture of toluene in methanol, to verify the instrument's proper operation. In addition, you can use the following procedures to test your instrument's reproducibility and sample carryover, and to troubleshoot and maintain the instrument in the future.

Tip Before beginning the performance verification runs, you might want to familiarize yourself with the autosampler's command center if you are controlling the autosampler in the Stand Alone mode.

For information on the autosampler's command center, see Chapter 3, "Keypad Controls."

This section contains the following topics:

Reproducibility

Use this procedure to measure your autosampler's injection reproducibility. In this procedure, you inject several volumes of a standard sample of 0.5 percent toluene/MeOH, determine the percent relative standard deviations (%RSD) for the injection volumes, and compare them with the desired results included in this section. The test takes approximately three hours. Retain these results for comparison to troubleshoot the injection system, if necessary.

Use the chromatographic conditions listed in Table 5 to test the autosampler's injection reproducibility.

Table 5. Injection reproducibility test method

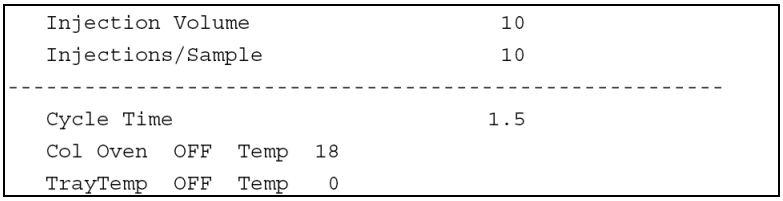
Column:	4.6 × 100 mm Spheri-5 RP18, 5 micron
Flow rate:	1.5 mL/minute
Mobile phase:	100% HPLC-grade methanol
Flush solvent:	100% HPLC-grade methanol
Test sample:	0.5% toluene in methanol (v/v)
Detection:	254 nm
Run time:	3 minutes

Test Setup

❖ To set up the test run

1. Fill five vials with the test sample and place them in tray positions A01 through A05.
2. Check for leaks.
3. Initialize your pump.
4. Prepare your detector for a run.
5. If you are not acquiring data through a data system computer, prepare your integrator.
6. Inspect all sample lines for bubbles. (Be sure to check the Teflon line that connects to port 3 of the injection valve.)
7. Do one of the following:
   - If the autosampler is set up as a stand-alone module, go to step 8.
   - If the autosampler is controlled from a data system, go to step 9.
8. For stand-alone operation, do the following:
   a. Create a file using the Injection menu parameters shown in Figure 37.

Figure 37. Injection menu performance verification parameters



b. Return to the Edit menu.

c. Access the More menu and verify that the Needle Height is set to 2.0. (Do not change any other parameters in this menu.)

d. Return to the main menu and load your file.

e. Press the SAMPLES key and set up a sample queue that contains five sample sets of one vial each. For each sample set, enter the following Sample menu values:

Inj/Sample **10**
Cycle Time **1.5**
No. Vials **1**

Vary the injection volume (Inj Vol) for each set using the values listed in Table 6.

Table 6. Reproducibility test sample queue parameters

Sample set	Inj Vol. (µL)	Sample vial
1	10	A01
2	1	A02
3	3	A03
4	7	A04
5	10	A05

f. Return to the main menu.

g. Flush the syringe with 500 µL of flush solvent. From the main menu, select **COMMANDS > Flush Sample Syringe**.

h. Press the RUN key.

9. For SpectraNet control, do the following:

a. Use the partial loop injection method for the autosampler and the method parameters listed in Table 5.

b. Set up a sequence that makes 10 injection per sample using the injection volumes and sample vial locations listed in Table 6.

c. Start your sequence run.

The autosampler makes 50 injections. The first group of ten injections is used to equilibrate the system, so do not use the data from these injections.

Test Results—%RSDs

Determine the percentage of relative standard deviations (%RSD) for the injection volumes and compare them with the desired results in Table 7.

Table 7. %RSD results for the reproducibility test

Volume (in µL)	%RSD
1	<1*
3	<0.7*
7	<0.5*
10	<0.5**

* Typical value

** Instrument specification

If your results are other than expected, see Chapter 7, "Troubleshooting."

Sample Carryover

Use this procedure to verify that the sample carryover is below the 0.01 percent specification for your autosampler. It requires the use of silicon septa, the appropriate volume of the required flush solvent, and clean (not previously used) blanks. The procedure minimizes the effects of sample concentration or chemistry, chromatographic conditions, detector linearity, or other hardware characteristics that might affect sample carryover.

This procedure is independent of sample- and system-specific characteristics. It reduces sample effects including extinction coefficients, chemical interaction with other sample or system components (the column), and sample degradation. It also minimizes system effects including detector linearity, pump and mixing anomalies, and column and guard-column contamination.

In this procedure you inject your sample using the push loop injection type, a subsequent blank (BLANK1), additional blanks to return the system to baseline, and then a standard (STANDARD) that is 0.01 percent of your sample. You determine the carryover percentage by multiplying the ratio of the carryover by the 0.01 percent specification:

$$\text{Carryover} = [\text{Peak Area}_{\text{BLANK1}}/\text{Peak Area}_{\text{STANDARD}}] \times 0.01\%$$

If desired, run this procedure three times to replicate the results.

Carryover Test Materials

Performing this procedure requires the following:

- Your sample
- 21 vials, septa, and caps
- Autosampler
- Any HPLC pump, detector, and data system

Carryover Test Sample Preparation

❖ **To prepare your sample and standard**

1. Place appropriate volumes of sample and starting mobile phase into a vial labeled SAMPLE.
2. Dilute your sample 10000-fold in starting mobile phase and pipette into a vial labeled STANDARD.

Example:

1. The sample is 100 mg protein in 1 mL phosphate buffer (SAMPLE).
2. Transfer 1 µL of SAMPLE into a 10 mL volumetric flask and dilute to volume with phosphate buffer (STANDARD).

Carryover Test Blank Preparation

❖ **To prepare your blanks**

1. Pipette fresh starting mobile phase into three sets of five vials.
2. Label vials as BLANK, BLANK1, BLANK2, BLANK3, and BLANK4 for each test set.

Carryover Test Procedure

❖ **To set up your system**

1. Prepare the appropriate mobile phase or phases.
2. Set up the appropriate chromatographic conditions for your sample.
3. Set up your integrator/data system so that it displays retention times and peak area information.

❖ To set up the autosampler injection parameters

1. Set the flush volume to **2000** µL and the injection type to **PushLoop**.

   Tip To set the flush volume and the injection type from the keypad controls, press the MENU key, and then select **Files > Edit > More**. Scroll down and select **Flush Volume**. Press the [+] or [–] keys to select **2000** µL. Scroll and select **Injection Type**, and then select **Push Lo**.

2. Enter an appropriate injection volume.

   Enter an injection volume no greater than 40 percent of the nominal sample loop size. For example, for a 100 µL sample loop, do not enter an injection volume greater than 40 µL.

   Tip To enter the injection volume from the keypad controls, press the MENU key, and then select **Files > Edit > Injection**. In the Injection Volume field, use the [+] and [–] keys to enter the injection volume.

3. Set the number of injections to **1**.

   Tip To enter the number of injections from the keypad controls, press the MENU key, and then select **Files > Edit > Injection**. In the Injections/Sample field, use the [+] and [–] keys to enter 1.

   IMPORTANT Make sure that the sample is soluble in the flush solvent. For best results, use the same solvent composition for the flush solvent and the mobile phase.

❖ To inject the sample set

1. Place the vials in the tray as follows:

   A01: BLANK
   A02: SAMPLE
   A03: BLANK1
   A04: BLANK2
   A05: BLANK3
   A06: STANDARD
   A07: BLANK4
   (Add additional Blanks as necessary to return the detector signal to baseline before replicating the test.)

2. Set up a sequence or an autosampler file to make one injection per vial.

3. (Optional but recommended) Set up two additional groups of seven vials to run the carryover test in triplicate.

4. Run your HPLC system using the appropriate chromatographic conditions for your sample.

Carryover Calculation

❖ To calculate the percentage of sample carryover

1. Divide the peak area of BLANK1 by the peak area of the STANDARD. This gives you the ratio of the carryover to the 0.01% specification.
2. Multiply this value by 0.01% to obtain the carryover percentage.

   Example:

   If the peak area for BLANK1 is 110958 counts,
   and the Peak area for the STANDARD is 656283 counts,
   the ratio of the carryover to the specification is 110958/656283 or 0.1691.
   % carryover = 0.1691 × 0.01%
   % carryover = 0.001691

Keypad Controls

This chapter introduces you to the autosampler's command center, provides you with three basic rules for using the keypad controls to navigate through the menu screens, and shows you how to make an injection using the keypad controls.

For more information on using the keypad controls, see Chapter 4, "Standalone Operation from the Command Center." If you are controlling the autosampler from a data system computer, refer to the Help provided with the data system for information on operating the autosampler.

Before you start this chapter, be sure that you read the Safety Information section and install your autosampler as described in Chapter 2, "Installation."

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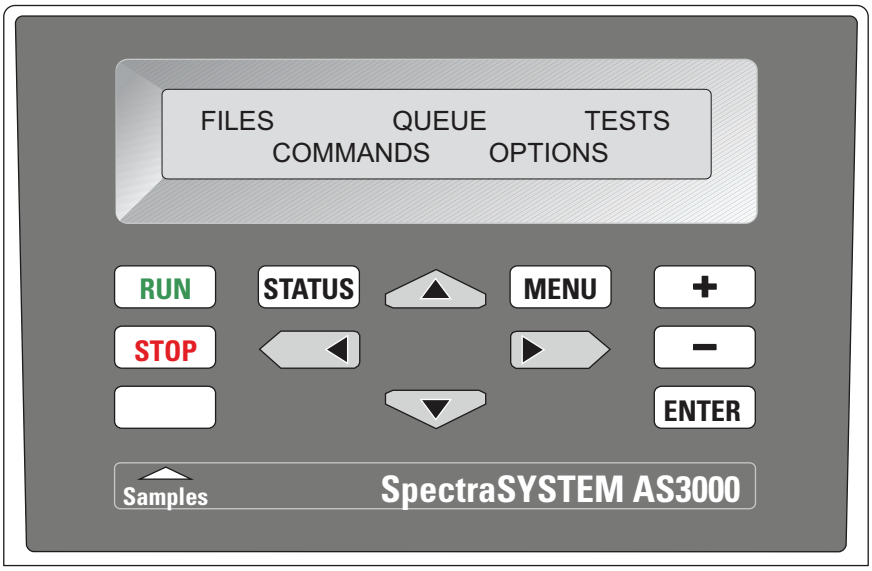
Note As you proceed through this chapter, you can explore the general architecture of your autosampler's menus and screens. You can also use this topic "SpectraSYSTEM AS3000 Menus" on page 151 as a guide.

To make your first injection, follow the procedures in "Standalone Operation from the Command Center" on page 63 or this two-page guide "Quick Start: Starting Runs from the Command Center" on page 155.

Command Center

The SpectraSYSTEM autosampler has a keypad and two-line display on the front panel (see Figure 38). This is the command center where you access menus and control the instrument's operations.

Figure 38. Command center for the SpectraSYSTEM autosampler



Navigating the autosampler menus assumes these three rules:

1. The arrow keys ▲, ▼, ◀, ▶ move the cursor in the direction printed on the key.

   Tip Press the MENU key to jump quickly to the top of the menu structure.

2. The shape of the cursor determines how you make a selection:

   - If a triangular cursor appears, press the ENTER key.
   - If a blinking square cursor ▒ appears, press the [+] or [–] keys to scroll up or down through preset choices, or to increase or decrease alphanumeric entries.

3. There are four ways to accept (and automatically save) an entry. Move the cursor out of the field by any of the following methods:

   - Pressing the ENTER key
   - Using the arrow keys
   - Pressing the MENU key
   - Pressing the STATUS key

   Note You cannot leave a menu if errors are present or if you have not completed all the necessary entries.

The autosampler display follows these conventions:

- Top-level menu choices appear in all-capital letters, except for Samples.
- A field's square cursor changes to an underscore cursor when you scroll through preset choices or enter numerical values and characters.
- A solid arrow, ▼, on the right side of some displays indicates that the current menu continues on additional screens. To access additional menu lines, press the DOWN ARROW key (▼) on the keypad.
- The last line of a longer menu is frequently a blank display line (without a solid arrow, ▼).

Keypad Functions

The keypad of each SpectraSYSTEM instrument consists of twelve keys. Four keys directly control the instrument's operation: RUN, STOP, STATUS, and, on the autosampler, a blank key called Samples. The remaining keys either access commands (MENU and ENTER), or are used to set parameters and move around the display keys (▲, ▼, ◀, ▶, [+], [–]).

Table 8 describes each key's function.

Table 8. SpectraSYSTEM keypad functions (Sheet 1 of 2)

Keys	Functions
RUN	Starts the run. The specific RUN operation depends on the contents of the sample queue: • If there are sample sets (groups of sample and associated calibration vials) in the sample queue, pressing the RUN key begins processing the first vial in the sample set. • If the sample queue is empty, pressing the RUN key prompts you to press the Samples key to display the Samples menu so that you can add sample sets to the sample queue.
STOP	Pauses the sample set currently running, cancels subsequent sample sets, and returns the autosampler to the initial run conditions. For more information on the STOP key's function, see Chapter 4, "Standalone Operation from the Command Center."
STATUS	Displays the Status screen. From the Status screen you can monitor the run in progress.
	Note Unlike the other SpectraSYSTEM instruments, on the autosamplers you can make changes to the run in progress from the Samples key rather than from the STATUS key (see below).

Table 8. SpectraSYSTEM keypad functions (Sheet 2 of 2)

Keys	Functions
Samples	The only variable key (unlabeled) in the whole SpectraSYSTEM family. (It has a different function on each of the SpectraSYSTEM modules.) On the autosampler, the blank key is the Samples key whose name appears on the nameplate below it. The Samples key displays the Samples menu, which you use to define the resources (samples, reagents, and standards) and assign a file to each sample set. From the Samples menu you also add sample sets to the sample queue. Because you manage resources and files from the Samples menu, the Samples key gives you the real-time editing capability provided by the STATUS key on the other SpectraSYSTEM instruments. That is, from the Samples menu you can make changes to the file that is currently running. By pressing the Samples key during a run, you can obtain information about, or edit the locations and injection volumes of, the sample and calibration vials. Chapter 4, "Standalone Operation from the Command Center," contains more information on the Samples key.
MENU	Displays the main menu (see Figure 39). For more information, see "Main Menu" on page 61.
ENTER	Accepts a selection or menu entry. The ENTER key also advances the cursor to a new field, either in the same line of the display or in the line below.
▲, ▼, ◀, ▶	The arrow keys (UP, DOWN, LEFT, or RIGHT) move the cursor in the direction indicated on the key. The UP and DOWN ARROW keys also move the cursor between menus and displays.
[+] and [–]	Scroll through a field's available choices, or changes the value of alphanumeric entries. Holding down either key continuously scrolls the list of choices forward or backward until you release the key. In fields that require alphabetical or numerical entries, the value of each digit is increased or decreased by one unit each time you press the [+] or [–] key. In fields that accept either numeric or character entries, such as the File Name field, the [+] and [–] keys scroll through the alphabet from A to Z; then through the numbers 0 to 9; and finally to a slash, hyphen, and blank space. In other fields, the [+] key advances you through a preset list of choices while the [–] key takes you back through the list.

Menus and Screens

Your autosampler has two kinds of displays: menus and screens. Menus require you to make selections or enter specific values. Screens display read-only information. The menu tree at the back of your manual (see "SpectraSYSTEM AS3000 Menus" on page 151) illustrates the structure and content of the autosampler's menus and screens.

Main Menu

The main menu (see Figure 39) is the top level of the menu structure. It gives you access to five other menus: FILES, COMMANDS, QUEUE, OPTIONS, and TESTS. To see the main menu, press the MENU key at any time.

Figure 39. Main menu accessed with the Menu key

The tasks associated with each menu follow:

Files menu	Edit, load, or delete files.
Queue menu	Edit or change the order of sample sets in the sample queue.
Tests menu	Run built-in instrument diagnostics.
Commands menu	Run built-in instrument routines.
Options menu	Set up or change the autosampler's configuration.

For more information on any of the autosampler's menus, see Chapter 4, "Standalone Operation from the Command Center," and Chapter 5, "Diagnostic Procedures."

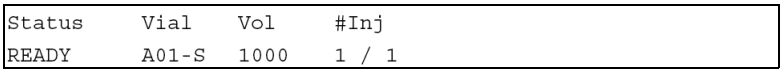
Status Screen

The Status screen automatically appears whenever you turn on the instrument or press the STATUS key. The Status screen (see Figure 40) displays the status of the sample queue, the current sample set, the current sample vial, and the total number of samples in the set.

Figure 40. Queue Status screen

Pressing the DOWN ARROW key (▼) displays the Injector Status screen (see Figure 41).

Figure 41. Injector Status screen



Pressing the DOWN ARROW key (▼) a second time displays the Oven Temperature Status screen (see Figure 42).

Figure 42. Oven Temperature Status screen

Chapter 4, "Standalone Operation from the Command Center," contains more information on the Status screen.

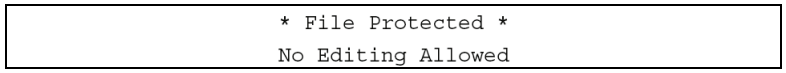
Messages

Three different kinds of messages can appear on your autosampler's display: user messages, confirmation messages, and error messages.

User Messages

User messages tell you about an existing instrument condition or ask for further actions. Some of these messages only appear on the display for three seconds. All user messages have a single asterisk before and after them. Figure 43 shows an example of a user message requiring further action.

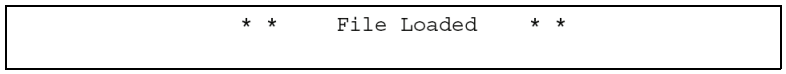
Figure 43. User message example



Confirmation Messages

A confirmation message appears for one second after the successful completion of an operation. All confirmation messages have a pair of asterisks before and after them. Figure 44 shows an example of a confirmation message.

Figure 44. Confirmation message example



Error Messages

An error message appears when the autosampler encounters an undesirable condition that prevents it from carrying out an operation. All error messages have a pair of exclamation points before and after them. An error message remains on the screen until you press a key. Figure 45 shows an example of an error message.

Figure 45. Error message example

Standalone Operation from the Command Center

This chapter describes how to use the autosampler's front panel controls to specify the injection parameters for your chromatographic methods and the vial locations for your injection sequences. Use these controls if you are not controlling the autosampler from a chromatography data system.

If you are controlling the autosampler from a chromatography data system, use the data system to create methods with the appropriate injection parameters and to create injection sequences with the appropriate vial locations. For information on controlling the SpectraSYSTEM autosampler from the data system, refer to the Help provided by the data system.

Before beginning this chapter, be sure that you have completed the Startup Checklist found in the beginning of this manual, installed your instrument according to the procedures described in Chapter 2, "Installation," and understand the keypad controls as described in Chapter 3, "Keypad Controls."

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Run Preparation

Using the front panel controls for the SpectraSYSTEM autosampler, you can process up to 39 non-priority sample sets (groups of sample vials and associated calibration vials) within a given run.

This section covers the following procedures, along with the instrument's file-management features.

IMPORTANT Be sure that your sample or samples are completely soluble in the mobile phase and that you have filtered your samples and solvents through a 0.45-micron filter. These techniques minimize sample precipitation in the lines and remove any particulate matter that could obstruct the flow through the autosampler's injection valve or the LC column. Fill the solvent reservoir with flush solvent.

Creating a File

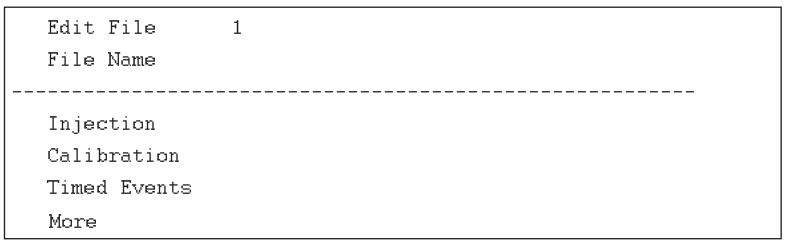
A file contains the autosampler parameters applied to a specific sample set.

You can create up to four unique files numbered 1 through 4. From the Edit menu, you can name your file and access additional menus to enter injection, calibration, timed events, and other run parameters.

Tip The following procedure describes how to create an edit file. For information on starting a run, see "Loading a File" on page 77.

Figure 46 shows the Edit menu. Selecting the Injection, Calibration, Timed Events, or More item opens an additional menu.

Figure 46. Edit menu



❖ To create a file

1. Specify a number and a name for the file.
   a. Press the MENU key.

      The main menu appears (see Figure 39 on page 61).

   b. Select **FILES > Edit**.

      The Edit menu appears (see Figure 46 on page 64).

   c. In the Edit File field, select **1**, **2**, **3**, or **4**.

      The file number identifies the file.

   The Edit File field indicates the number of the selected file. File 1 automatically appears in this field when the Edit menu is first displayed. If you want to edit a different file, press the [+] and [–] keys to select a different file number.

2. (Optional) In the File Name field, enter a name for this file.

   When you first display the Edit menu, the File Name field contains eight blank characters. Press the [+], [–], UP ARROW ▲, and DOWN ARROW ▼ keys to create a file name using the following characters: A to Z, 0 to 9, \, blank, dash, or hyphen.

3. Select the injection type as follows:
   a. Press the DOWN ARROW key (▼) until the cursor reaches the More menu item, and then press ENTER.

   b. Press the DOWN ARROW key (▼) until the cursor reaches the Injection Type menu item, and then press ENTER.

   c. Select the injection type.

4. Make the appropriate entries in these menus:
   - "Injection Menu" on page 66
   - "Calibration Menu" on page 69
   - "Timed Events Menu" on page 72
   - "More Menu" on page 74

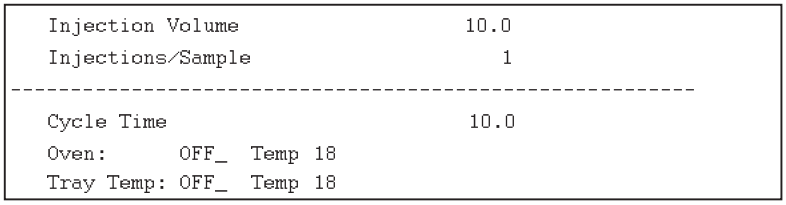
   When you exit the file, the autosampler automatically saves the file.

Injection Menu

Use the Injection menu to specify the injection volume, the number of injections per sample, the time between injections, and the temperature of the autosampler's controlled temperature zones.

Figure 47 shows the Injection menu and Table 9 describes the parameters in the Injection menu.

Figure 47. Injection menu (AS3000 autosampler)



❖ To specify the injection parameters

1. From the main menu, choose **File > Edit > More**.
2. Select the appropriate injection type (see "More Menu" on page 74).
3. Choose **File > Edit > Injection**.
4. In the Injection Volume field, do one of the following:
   - For the full-loop injection type, select the appropriate loop size.

     The selections depend on the syringe size (see "Injection Volume or Loop Size" on page 67).
   - For the pull-loop injection type, enter an injection volume from **0.1** to **212** µL for the 250 µL syringe.
   - For the Push Lo injection type, enter an injection volume from **0.1** to **10** µL.
   - For the Push Hi injection type, enter an injection volume from **0.1** to **200** µL.
5. In the Injections/Sample field, enter the number of injections that you want the autosampler to make per sample vial.
6. In the Cycle Time field, enter the initial time between injections.
7. In the Oven field, select **On** to activate temperature control, and then enter the appropriate column oven temperature from 15 to 80 °C, in one-degree increments.
8. In the Tray Temp field, select **On** to activate temperature control, and then enter the appropriate tray compartment temperature from 0 to 60 °C, in one-degree increments.

   After you complete the injection parameters, the cursor moves to the Calibration menu item.

Table 9. Injection menu parameters (Sheet 1 of 2)

Parameter	Description	
Injection Volume or Loop Size	Specifies the injection volume or the loop size. The selections or range depends on the injection type and the syringe size.	
	Injection Type	Selection or range
	Loop Size (Full-loop injection)	The selections depend on the syringe size.
	Syringe size	**Selections**
	250 µL	5, 10, 20, 50, and 100 µL
	500 µL	5, 10, 20, 50, 100, and 200 µL
	1000 µL	5, 10, 20, 50, 100, 200, and 500 µL
	2500 µL	5, 10, 20, 50, 100, 200, 500, and 1000 µL
	5000 µL	5, 10, 20, 50, 100, 200, 500, and 1000 µL
	IMPORTANT Be sure that the Loop Size field value reflects the size of the sample loop.	
	Pull	The maximum injection volume depends on the syringe size.
	Syringe size	**Maximum injection volume**
	250 µL	212 µL
	500 µL	461 µL
	1000 µL	956 µL
	2500 µL	1500 µL
	5000 µL	1500 µL
	Push Hi	Range: 0.1 to 10.0 µL
	Push Lo	Range: 1 to 200 µL
	IMPORTANT For a variable-loop injection, limit the injection volume to approximately one-half the sample loop size.	
Injections/Sample	Specifies the number of injections to be made from each vial (1 to 99).	

Table 9. Injection menu parameters (Sheet 2 of 2)

Parameter	Description
Cycle Time	Specifies the time (0 to 655.3 minutes) between injections (from the injection to the end of the run). In calculating this value, be sure to allow enough time for the column to equilibrate (if you are running a gradient), for all peaks to elute from the column, and for the integrator to print any post-run reports and perform any post-run calculations. The autosampler is capable of making an injection approximately every 90 seconds, depending on the injection and flush volumes selected. If a Pump Ready contact closure controls your cycle time, you may set the Cycle Time to 0.0.
	Tip The default flush volume is 400 µL so that the total injection cycle time (including the flush cycle) is approximately two minutes.
Column Oven	Appears only if the Column Oven is set to ON in the Configurations menu. Selections: On or Off Temperature range: 15 to 80 °C, in one-degree increments
	Tip • You can specify temperatures starting at 5 °C above ambient. • When you set the oven's state to On, the oven does not activate (turn on) until you load your file. • The autosampler automatically delays the start of the run until the oven reaches the set temperature. Once activated, the oven requires about 15 minutes to reach a temperature of 70 °C. To save time, you might want to load your file so that the oven can be warming up while you are completing your run preparation and entering the file parameters for your run. For more information on loading your file, see "Loading a File" on page 77.
Tray Temperature Control	Appears if the Tray Temperature is set to ON in the Configurations menu. Selections: On or Off Temperature range: 0 to 60 °C, in one-degree increments For more information, see "Autosampler Components" on page 2.
	Tip • When you set the tray temp state to On, the tray temperature controller does not activate (turn on) until you load your file. • Once activated, if empty, the tray compartment requires about 30 minutes to reach a temperature of 0 °C and about 10 minutes to reach a temperature of 60 °C. To save time, you might want to load your file so that the tray compartment can be warming up while you are completing your run preparation and entering the file parameters for your run. For information on loading your file, see "Loading a File" on page 77.

Calibration Menu

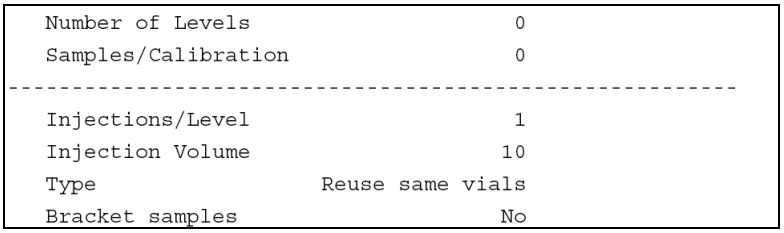
Use the Calibration menu to set up the calibration parameters, such as the number of calibration levels and the number of sample injections between calibration sets, for your sample set.

Note You specify the numbers of samples per sample set and the vial locations for samples and calibration standards in the Samples menu.

Figure 48 shows the Calibration menu and Table 10 describes the parameters in the Calibration menu.

Note The data system that you use to acquire and process your data files does not recognize the information that you enter in the Calibration menu. To process your data files, you must create an appropriate processing sequence from your data system that contains the appropriate sample and calibration information.

Figure 48. Calibration menu



❖ To specify the injection settings for the calibration vials

1. From the main menu, choose **File > Edit > Calibration**.
2. In the Number of Levels field, select the number of calibration levels for your application.
3. In the Samples/Calibration field, select the number of sample vials between calibration vials.
4. In the Injections/Level field, select the number of injections that you want the autosampler to make from each calibration vial.
5. In the Injection Volume or Loop Size field, select the injection volume for the calibration vials or the loop size (full-loop injection type).

   For information on the injection volume range or loop size selections, see "Injection Volume or Loop Size" on page 67.

6. In the Type field, specify whether you want the autosampler to reuse one set of vials for the calibration standards or a new set of calibration vials for each calibration set:

   - To specify a single location for the calibration vials, select **Reuse Same Vials**.

     If you select Reuse Same Vials, load only one set of calibration vials in the sample trays. Specify the location of the first calibration vial in the Samples menu. The autosampler determines the number of calibration vials based on your entry in the Number of Levels field (see Table 10).

   - To specify a new set of calibration vials for each calibration set, select **Intersperse Vials**.

     If you select Intersperse Vials, load the trays with a set of calibration vials for each calibration set as specified in the Samples/Calibration entry. Specify the location of the first calibration vial in the Samples menu. The autosampler determines the total number of calibration vials based on your entries in the Number of Levels, Samples/Calibration, and Bracket Samples fields (see Table 10).

7. In the Bracket Samples field, select **Yes** or **No**.

   After you complete the injection parameters, the cursor moves to the Timed Events menu item.

Table 10. Calibration menu parameters (Sheet 1 of 2)

Parameter	Description
Number of Levels	Specifies the number of calibration levels (0 to 9). The autosampler automatically copies this value to the Calib 1Vial 1 (of *X*) field, in the Samples menu, where *X* equals the number of levels. (For more information, see Calib Vial 1 (of X) on page 79.) A value of zero in the Number of Levels field indicates that there are no calibration injections for the specified sample set.
Samples/Calibration	Specifies the calibration interval (the number of sample vials to be injected between each recalibration).
Injections/Level	Specifies the number of injections (1 to 99) from each calibration level (0 to 9).
Injection Volume or Loop Size	See the information on the Injection Volume or Loop Size parameter in the Injection menu (Table 9 on page 67).

Table 10. Calibration menu parameters (Sheet 2 of 2)

Parameter	Description	
Type	Specifies one of two types of patterns for determining the location of the calibration vials:	
	Pattern	**Use**
	Reuse Same Vials	Programs the autosampler to use the same calibration vials each time a recalibration is made. When a calibration is required, the autosampler goes to the first calibration-vial position specified in the Sample menu and injects all the calibration vials. It then injects the number of samples indicated in the Calibration menu for Samples/Calibration.
	Intersperse Vials	Programs the autosampler to use each calibration vial only once. You must manually intersperse your calibration vials between your sample vials within the sample set. The autosampler makes injections in tray-placement order. You specify the location of the first calibration vial in the Samples menu. The autosampler uses the value that you enter in the Samples/Calibration field to determine the location of subsequent calibration vials.
Bracket Samples	Specifies whether or not you want to automatically end the sample set with a recalibration. If you select Yes, the autosampler processes the last sample and then repeats the calibration series specified for the sample set. If you select No, the autosampler stops and returns home after it injects the last sample.	

Table 11 shows the injection sequence when you select either the Reuse Same Vials or the Intersperse Vials calibration routines, as well as the injection sequence when you select Bracket, for the following sample set:

- Number of samples: 4
- Calibration levels: 2
- Number of samples between each calibration set: 2
- First calibration location: A01
- First sample location: A03

Use the Calibration menu to specify the number of calibration levels per calibration set and the number of samples between calibration sets. Use the Samples menu to specify the number of samples and the vial locations for samples and calibration standards.

Note The autosampler does not send the calibration and sample information to your integrator or data system. To analyze the results of the injection set, you must reenter the calibration and sample sequence into your data processing system.

Table 11. Comparison of the total number of vials used for the calibration routine selections

Injection	Reuse vials	Reuse vials and bracket	Intersperse vials	Intersperse vials and bracket
C	A01	A01	A01	A01
C	A02	A02	A02	A02
S	A03	A03	A03	A03
S	A04	A04	A04	A04
C	A01	A01	A05	A05
C	A02	A02	A06	A06
S	A05	A05	A07	A07
S	A06	A06	A08	A08
C	N/A	A01	N/A	A09
C	N/A	A02	N/A	A10
Total # of vials	**6**	**6**	**8**	**10**

Timed Events Menu

Use the Timed Events menu to create a time program to turn on/off external devices (such as column-switching valves and fraction collectors) at preset times during your run. For timed events, the autosampler uses pins 9 to 12 from its 12-pin terminal. For information on connecting external devices to your autosampler, see "Stand Alone Mode" on page 39.

Figure 49 shows the autosampler's Timed Events menu.

Figure 49. Timed Events menu

```
  Time    TF1     TF2     TF3     TF4
  0.00    Lo      Lo      Lo      Lo
-------------------------------------------------------
  0.50    Hi      Lo      Lo      Lo
  2.00    Lo      Hi      Lo      Lo
```

The Timed Events menu holds a maximum of nine lines, each of which sets Hi or Lo for one or more timed event outputs at the times specified. When the TF terminal is set to Hi, it presents a +5 V signal.

Figure 50 shows a timed events program that does the following:

- At 0.5 minutes, turns on the TF2 terminal.
- At 1.00 minutes, turns off the TF2 terminal and turns on the TF3 terminal.
- At 2.00 minutes, turns on the TF2 terminal and turns off the TF3 terminal.

Figure 50. The timed events program described above

Time	TF1	TF2	TF3	TF4
0.00	Lo	Lo	Lo	Lo
0.50	**Lo**	**Hi**	**Lo**	Lo
1.00	Lo	Lo	Hi	Lo
2.00	Lo	Hi	Lo	Lo

Note The Run-time clock stops and resets at the completion of the Cycle Time. Time lines longer than the Cycle Time value are never activated.

❖ To add a line to the end of the timed events program

1. Press the DOWN ARROW key (▼) to move the cursor to the blank line after the last displayed line.
2. Press the [+] key to copy the last existing line with a new time incremented by one minute.
3. Use the arrow keys and the [+] and [–] keys to change the time and set the time functions' states (Hi/Lo).

❖ To delete a line from the timed events program

1. Move the cursor to the Time field in the line to be deleted.
2. Hold down the [–] key until the value is zero (0.00).
3. Press the [–] key once to erase the line.

If you are deleting a line from the middle of a timed events program, the blank line remains until you move the cursor to another line.

Note If you delete both of the visible lines on the display, both lines disappear, but the cursor remains. Press the UP and DOWN ARROW keys (▲ or ▼) to move to the remaining lines in the program.

❖ To insert a line between two existing lines in the timed events program

1. Move the cursor to the blank line at the end of the program.
2. Enter a time that falls between two existing times.
3. Move the cursor off the new line. The autosampler automatically inserts the new line.
4. Move the cursor to the newly inserted line or lines and set the Timed Events Outputs' states as desired.

More Menu

Use the More menu (Figure 51) to enter the remainder of your run parameters.

Figure 51. More menu

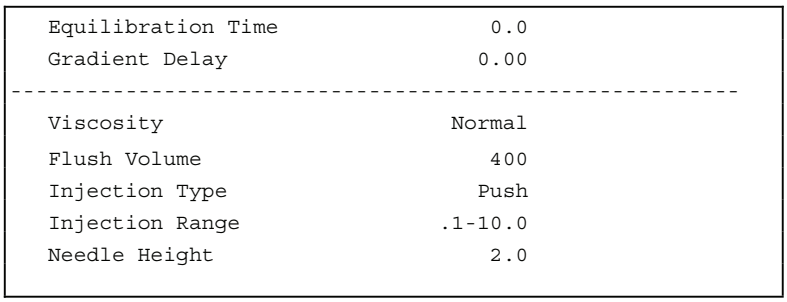


Table 12 describes the More menu parameters.

Table 12. More menu parameters (Sheet 1 of 3)

Parameter	Description
Equilibration Time	Specifies the length of time (in minutes) required for the column or LC system to equilibrate. The equilibration time allows time for the column to equilibrate prior to the first injection of a new sample set. It is only in effect before the first injection. The default value of 0.0 is a good starting value for many applications.
Gradient Delay	Specifies the amount of time required to allow the mobile phase to travel from the pump's proportioning valve to the column. Use this field only if you are running a gradient. To determine the gradient delay value, you must calculate the pre-column volume, which is best done during installation. To calculate your pre-column volume and gradient delay value, see "Gradient Delay" on page 99.
Viscosity	Specifies the sample viscosity (Normal, Medium or Viscous). The viscosity setting affects the sample syringe draw-rate. A higher viscosity setting automatically decreases the syringe draw-rate. **Tip** Use the Normal setting for running most reversed-phase applications.
Flush Volume	Specifies the flush volume. Range: 200 to 5000 µL Default: 400 µL

Table 12. More menu parameters (Sheet 2 of 3)

Parameter	Description	
Injection Type	For a description of each injection type and the excess sample volume required for injection, see "Injection Types" on page 4. The optimum injection type depends on the amount of sample you have and the degree of precision desired. Choose from four different injection types:	
	Full-loop	Full- or fixed-loop injection is useful when you want maximum precision and have unlimited sample. The actual injection volume depends on the loop size. **Note** If you select full-loop injection, the Injection Volume field in the Injection menu changes to Loop Size and the Injection Volume field in the Calibration menu does not appear.
	Pull-loop	Pull-loop injection (traditional variable-volume injection) is useful when sample conservation is important and when large injection volumes (> 100 µL) are required. **Tip** Use a sample loop that is at least twice the size of the largest injection volume. For example, use a 200 µL sample loop or larger to inject 100 µL.
	PushLoop (Push Hi)	PushLoop injection (variable-volume method) gives the precision of fixed-loop injection with limited amounts of sample.
	PushLoop (Push Lo)	Use the PushLoop (Push Lo) method for smaller injection volumes (< 10 µL).

Table 12. More menu parameters (Sheet 3 of 3)

Parameter	Description	
Injection Range	The Injection Range field displays the injection volume range, depending on the Injection Type that you select.	
	Injection type	**Range**
	Full-loop	Displays the range: 5 to 1000. During a full-loop injection, the autosampler overfills the sample loop. The actual injection volume equals the physical size of the sample loop, which is ± 20 percent of the loop's nominal size.
	Tip When you select the Full Loop injection type, the Edit > Injection > Injection Volume field changes to Loop Size. The Loop Size selections depends on the configured syringe size.	
	Pull-loop	Displays the range: 1 to 1500 µL. Specify the injection volume in the Injection Volume field of the Injection menu.
	PushLoop (Push Lo)	Displays the range: 0.1 to 10.0 µL. Specify the injection volume in the Injection Volume field of the Injection menu.
	PushLoop (Push Hi)	Displays the range: 1 to 200 µL.
	Tip For the variable-volume injection types, specify the injection volume in the Injection Volume field of the Injection menu.	
Needle Height	Controls the distance between the needle tip and the bottom of the vial. Range: 0.0 to 20.0 mm Default: 2.0 mm	

Saving a File

The autosampler automatically saves your file. Once you have filled in all of the Edit menu parameters, you have finished creating your edit file. Exiting the Edit menu automatically saves your file but does not load it. For more about file management, see "File Management" on page 90.

Note Pressing the RUN key after creating your file does not start your run. You must first add it to the sample queue. For more information, see "Building a Sample Queue" on page 78.

Loading a File

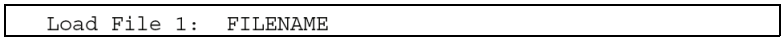
Use the Load function when you want to run a single sample set manually, for example, to turn on your oven or tray heater/cooler.

❖ To load an Edit file

1. From the main menu, select **FILES > Load**.

   The Load menu appears.

   Figure 52. Load File field

2. Do one of the following:

   - To load the last file you edited, press the [+] and [–] keys to select its file number and press the ENTER key.

     –or–

   - To load a different file, select the correct file number and then press the ENTER key.

   The **File Loaded** message appears. Once a file is loaded, it becomes your run file.

You use the Load function to run a single sample set; however, if you want to run a group of sample sets, press the RUN key from the sample queue. Once you press RUN, the autosampler automatically loads each file into the queue before running it. You do not need to manually load each file. For more information on running your samples from the sample queue, see "Building a Sample Queue" on page 78

Building a Sample Queue

The sample queue is the list of sample sets and associated files to be run. Building your sample queue requires adding each item to the queue from the Samples menu and then arranging those items in the desired run order from the Queue menu. If you run a group of files from the sample queue, the SpectraSYSTEM application automatically loads each file before it is run.

To build a sample queue, follow these procedures:

- "Adding Items to the Sample Queue" on this page
- "Changing the Order of Sets in the Queue" on page 80
- "Deleting Sets from the Queue" on page 80
- "Managing a Priority Set in the Queue" on page 81

Adding Items to the Sample Queue

❖ To add an item to the sample queue

1. Press the Samples key to access the Samples menu.
2. Enter each of the values shown in the Samples menu (see Figure 53) and described in Table 13.

Tip Since the method downloads most of the values automatically, the only values that require editing are those in the File Number and Vial Location fields.

Figure 53. Samples menu

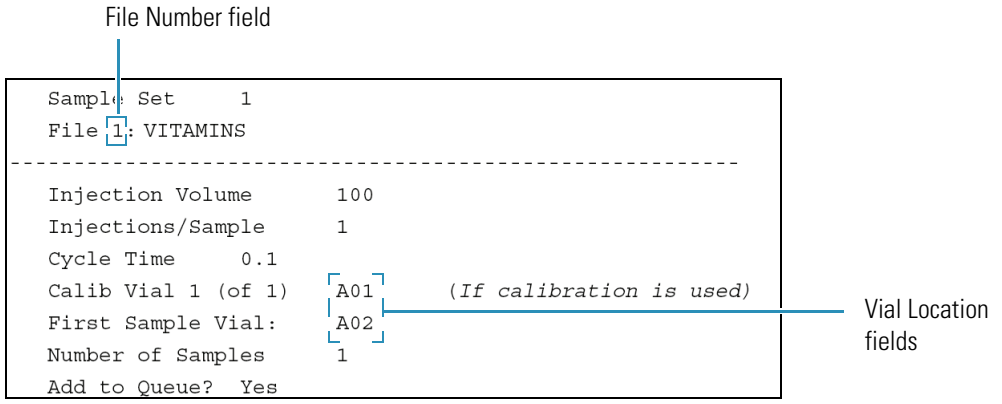


Table 13. Samples menu parameters

Parameter	Description
Sample Set	Specifies the sample set you want to add to the queue. You can add the sample set only once to the sample queue. You can load a maximum of 39 sets into the sample queue, plus one priority set.
File	Specifies the file number to be applied to the specified sample set. The file parameters determine the sequence of Sample Set prompts. Range: 1 to 4
Injection Volume, Injections/Sample, and Cycle Time	These values are copied from the Injection menu of the associated file.
Calib Vial 1 (of *X*)	This value is copied from the Calibration menu of the associated file. Specifies the location of the first vial in the calibration series. *X* is an indicator of the number of calibration levels specified in the file. Range: A01 to C35 **Tip** The vial position automatically wraps.
First Sample Vial	Specifies the location of the first sample vial. Range: A01 to C35
Number of Samples	Specifies the number of sample vials in the sample set. Range: 1 to 105
Add to Queue	Selecting Yes and pressing the ENTER key adds the selected sample set to the queue. You can add each set only once to the sample queue. Selecting No returns you to the Status menu.

3. After filling in the Samples menu for the first sample set, press the Samples key again. This returns you to the top of the menu where you can add the next sample set.

Figure 54 shows a Queue menu with three sample sets.

Figure 54. Queue menu with no priority-vial sample sets in the queue

Order	File Name	Set
1	1:TEST	3
2	2:SAMPLE1	4
3	3:AA1	5

Changing the Order of Sets in the Queue

❖ To change the order of the items in the queue

1. Move the cursor to the line where you want to change the order.
2. Increment or decrement the set to the new Order value.

   For example, to run sample set 5 before sample set 3, move the cursor to the order column of the sample-set 5 line, and decrement the Order value to 1 (see Figure 55).

Figure 55. Queue with three sequential sets

Order	File Name	Set
1	1:TEST	3
2	2:SAMPLE1	4
3->1	**3:AA1**	**5**

Changing the run order of set 5 from third to first

The lines rearrange themselves after you exit the field (see Figure 56).

Figure 56. Queue with reordered sets

Order	File Name	Set
1	**3:AA1**	**5**
2	1:TEST	3
3	2:SAMPLE1	4

Tip You can only change Order 1 if the sample set is not active—that is, when you press the STATUS key, the QStatus column reads IDLE.

Deleting Sets from the Queue

❖ To delete an item from the sample queue

1. Move the cursor to the line containing the item you want to delete.
2. Decrement the Order number to **0** and then blank.

   Moving the cursor to another line deletes the blank line from the display.

Managing a Priority Set in the Queue

A priority set is inserted into the active set and its samples run before other samples in the active set. It must be assigned to the active file. It appears in the Queue menu with a P in the Set field (see Figure 57). You cannot assign or change its priority status from the Queue menu. A priority set is automatically deleted from the queue upon completion of the active set or when the queue (QStatus) returns to its IDLE state (if you press the STOP key). For more information on running priority vials, see "Priority Sample Sets" on page 87.

Figure 57. A sample queue that contains a priority set

```
  Order   File Name       Set
  1       1:SAMPLE1       P
-----------------------------------------------------------
  1       1:SAMPLE1       4
  2       3:AA1           5
```

Entering Your Other LC Parameters

Prepare your other LC modules for a run and enter the run parameters for your pump, detector, and data-output device (recorder, integrator, or data system). Before you start your run, complete these tasks:

- Initialize your pump and degas your solvents as required for your application.
- Be sure your detector is stabilized. Do not start your analysis until you achieve a stable baseline.
- Set up your data-output device.

For more information on connecting your autosampler to other LC modules, see Chapter 2, "Installation."

Starting a Run by Pressing the Run Key

Once you have created and loaded your file, added your sample set or sets to the sample queue, and installed your sample trays, you are ready to start your run.

❖ **To start a run**

1. Load your sample and calibration vials into the trays and install the trays into the autosampler tray compartment (see "Installing the Sample Trays" on page 27).

   The tray and vial positions from left to right and front to back of the tray compartment are A01 to C35.

2. Press the RUN key.

Depending on whether you have loaded sample sets into the sample queue, the status screen displays the following:

- If there are sample sets in the sample queue, the RUN status for the sample queue is displayed on the Status screen (see Figure 58).
- If the queue is empty, the autosampler prompts you to access the Samples menu so that you can add sample sets to the queue. After you have added sets, press the RUN key to start your run.

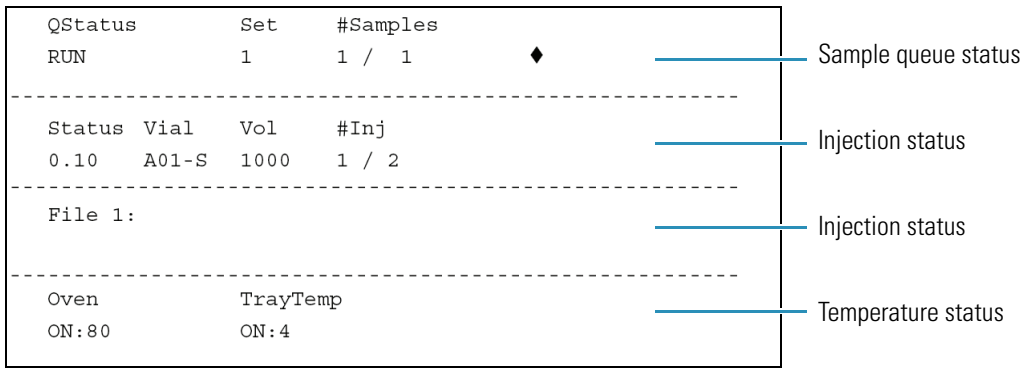
Monitoring the Run Status

When you press the RUN key, the autosampler begins processing vials when the temperature for the column oven, tray compartment, or both reaches the set point; the equilibration delay time (entered in the More menu) has elapsed; and the tray compartment door is closed. If the autosampler is connected to an LC pump through a contact closure connection, the autosampler also waits until the pump is ready.

During the run, the Status screen appears (see Figure 58).

Note You can change the ready requirements by choosing Options > Ready Participation and selecting Enable or Disable in each field. See "Ready Participation" on page 48.

Figure 58. Status screen



The Status screen gives you four kinds of information (from top to bottom):

- "Sample Queue Information" on page 83
- "Injection Status" on page 84s
- "File Status" on page 85
- "Temperature Status" on page 85

Sample Queue Information

Information on the sample queue is presented in the first two lines of the Status screen (see Table 14).

Table 14. Sample queue status fields

Field	Description	
QStatus	Displays the status of the queue (IDLE, PAUSE, RUN, EQUIL, ABORT).	
	Message	**Meaning**
	IDLE	Indicates that no sample sets are currently being processed in the queue.
	Tip The IDLE status does not indicate if sets are loaded into the queue. To check the contents of the sample queue, press the MENU key and select **QUEUE**.	
	PAUSE	Indicates that a sample set is loaded, but that processing has been suspended. If you pause the queue (by pressing the STOP key once), you can either resume processing with the current or subsequent vial, or cancel processing of the current sample set (see "Stopping a Run" on page 88).
	RUN	Indicates that a sample set in the queue is being processed.
	EQUIL	Indicates that the autosampler is waiting for the equilibration delay (temperature zone and initial conditions) to be reached before it starts processing the current sample set. This status is displayed until the oven and tray compartment reach their set temperatures, the equilibration time has elapsed, or both.
	ABORT	Indicates that the processing of the current sample set has been stopped (by pressing the STOP key and selecting ABORT from the Abort/Continue menu) or that a system error has occurred. The QStatus displays ABORT until the autosampler has returned the active vial to the sample tray and flushed all the lines. For more information about Abort, Continue, and Pause, see "Stopping a Run" on page 88.
Set	Displays the number of the sample set currently being processed (1 to 39, P). If the queue is not running (the QStatus reads other than RUN or PAUSE), the set field is blank.	
#Samples	Displays the number of the sample currently being processed and the total number of samples in the set: current sample number/total of samples. For example, if the autosampler is processing sample 50 in a set of 120 samples, the #Samples field reads 50 / 120.	
	Tip The total does not indicate the total number of vials or the total number of injections in the set. Calibration vials are not included.	

Injection Status

After you start a run, pressing the DOWN ARROW key (▼) displays the injection status screen that contains information on the injection in progress (see Table 15).

Tip The Status Screen updates every two seconds.

Table 15. Injection status parameters (Sheet 1 of 2)

Field	Description	
Status	Displays the progress of the current injection. A time in minutes in the Status field indicates that the queue is running (RUN) and that the current sample has been injected. In addition to the run time, the status field displays these messages.	
	Message	**Meaning**
	RDY	Indicates that the autosampler is ready for an injection (all temperature zones and gradient conditions have been reached), but that no injection is currently in progress.
	NOTRDY	Indicates that the autosampler is not ready for an injection. NOTRDY might indicate that the temperature zones are not stabilized, the door is opened, or the Pump Ready contact closure is in the wrong state.
	GRAD	Indicates that the autosampler is waiting for the set gradient conditions to be reached—that is, for the gradient delay time set in the More menu to elapse.
	HOLD	Indicates that an injection hold is in effect as determined by the Inject Hold input or as set in the Input Polarity menu.
	ABORT	Indicates that the current injection has been stopped in one of two ways: • By pressing the STOP key to stop the Queue and selecting ABORT from the Abort/Continue menu –or– • By an error condition occurring During the ABORT sequence, the autosampler returns the current vial to the sample tray and flushes the lines.
	DOOR	Indicates that the door is open. The autosampler automatically moves the arm to the home position to provide easy access to the sample tray. The arm automatically resumes where it left off when the door is closed.
Vial	Indicates the position and type of vial (S for sample vial, C for calibration vial) currently being injected. If no injection is in progress (the Status reads NOTRDY), this field is blank.	

Table 15. Injection status parameters (Sheet 2 of 2)

Field	Description
Vol	Displays the injection volume for the current vial. If the current vial is a sample vial, this field indicates the Injection Volume value entered in the Injection menu. If the current vial is a calibration vial, this field displays the Injection Volume value entered in the Calibration menu.
#Inj	Displays a fraction that indicates the current injection number over the total number of injections requested for the current vial: current injection number/injection per vial. For example, if the autosampler is performing the third of five injections, the #Inj field reads 3 / 5.
File #	Pressing the DOWN ARROW key (▼) displays the number and name of the file assigned to the sample set currently being processed.
Temperature Status	Pressing the DOWN ARROW key (▼) accesses the Temperature Status screen, which displays the actual temperature of the column oven and the tray compartment. The temperature is displayed, even if this option is turned off (not set). OFF indicates that an option is installed but not turned on in the current file's Injection menu as shown in XXX.
	Tip To check the set temperature for the oven, you can return to the Edit menu and view the information for the appropriate file as long as you have not loaded that file into the Queue. If you want to check any experimental parameters after loading your run file, make a copy of your file that you can review before loading it.

File Status

After you start a run, press the DOWN ARROW key (▼) two times to display the file status.

The File field displays the number and name of the file assigned to the sample set currently being processed.

Temperature Status

After you start a run, press the DOWN ARROW key (▼) three times to display the status of the column oven and tray compartments (see Table 16).

Table 16. Temperature status

Field	Description
Oven	
ON or OFF	OFF indicates that the oven temperature control is turned off.
Temperature	Indicates the actual temperature of the column oven compartment.
Tray	
On or Off	OFF indicates that the tray compartment temperature control is turned off.
Temperature	Indicates the actual temperature of the column oven compartment.

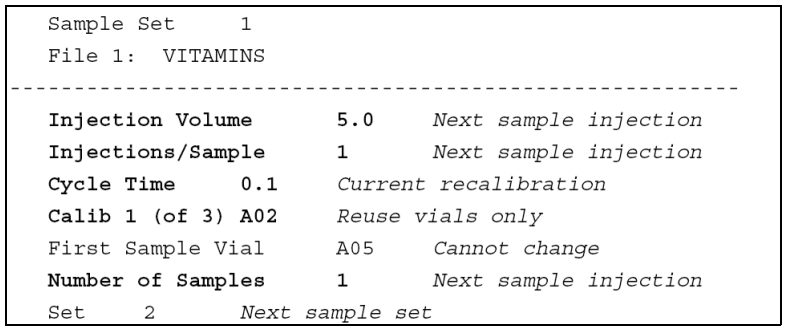
To check the set temperature of the column oven or tray compartment, you can return to the Edit menu and view the information for the appropriate file as long as you have not loaded that file into the Queue. If you want to check any experimental parameters after loading your run file, make a copy of your file that you can review before loading it.

Changing the Run in Progress

Once a run has started, use the Samples menu if you want to change the injection volume, cycle time, calibration interval, or set assignments for the sample set currently in progress.

Figure 59 lists the changeable parameters in bold-face type and indicates when those changes take effect. With the exception of the Cycle Time and Calib 1(of *X*) values, changes made from the Samples menu take effect on the next sample injection. Changes to the Cycle Time take effect on the current sample injection; changes to the Calib 1(of *X*) field take effect on the next recalibration. Samples menu changes remain in effect until the next sample set.

Figure 59. Samples menu



Note Changing one vial location does not affect other vial locations.

This section contains the following topics:

Priority Sample Sets

A priority sample set is one that you can insert into the currently active sample set at any time. It shares the same file and resources as specified for the currently active set.

Priority sample sets are like other sample sets with several important exceptions:

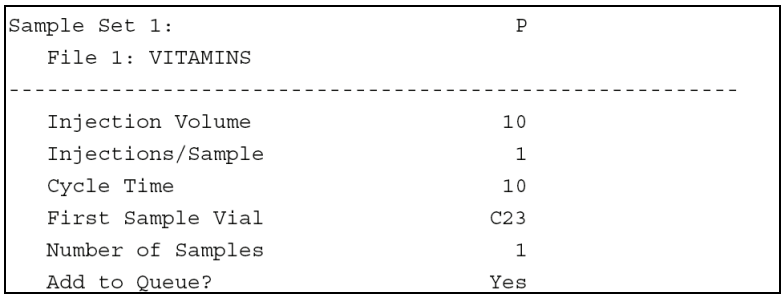
- You assign their priority set number (P) in the Samples menu (not in the Queue menu).
- You cannot change the order of priority sample sets once they are loaded into the sample queue.
- If you want to run priority sample sets, you must select Reuse Same Vials as the calibration routine type. (If you select Intersperse Vials, the autosampler does not distinguish whether a subsequent vial is a calibration vial or a sample vial. As a result, it cannot resume the calibration routine after the priority sample set is processed.)
- Since a priority set is inserted into the currently active set, you cannot edit or change the file between the currently active set and the priority set.

❖ To insert a priority sample set into the sample queue

1. Press the Samples key.
2. Press the [+] and [–] keys to change the Set field value to **P**.

   Figure 60 shows a priority sample set with P in the Set field.

   Figure 60. Samples menu with a priority sample set



3. Fill in the other file parameters as you would for any other sample set.
4. Add the priority set to the queue.

   The autosampler assigns a P (priority) order to the priority set as shown in the Queue menu (see Figure 61):

Figure 61. Queue menu with one priority sample set

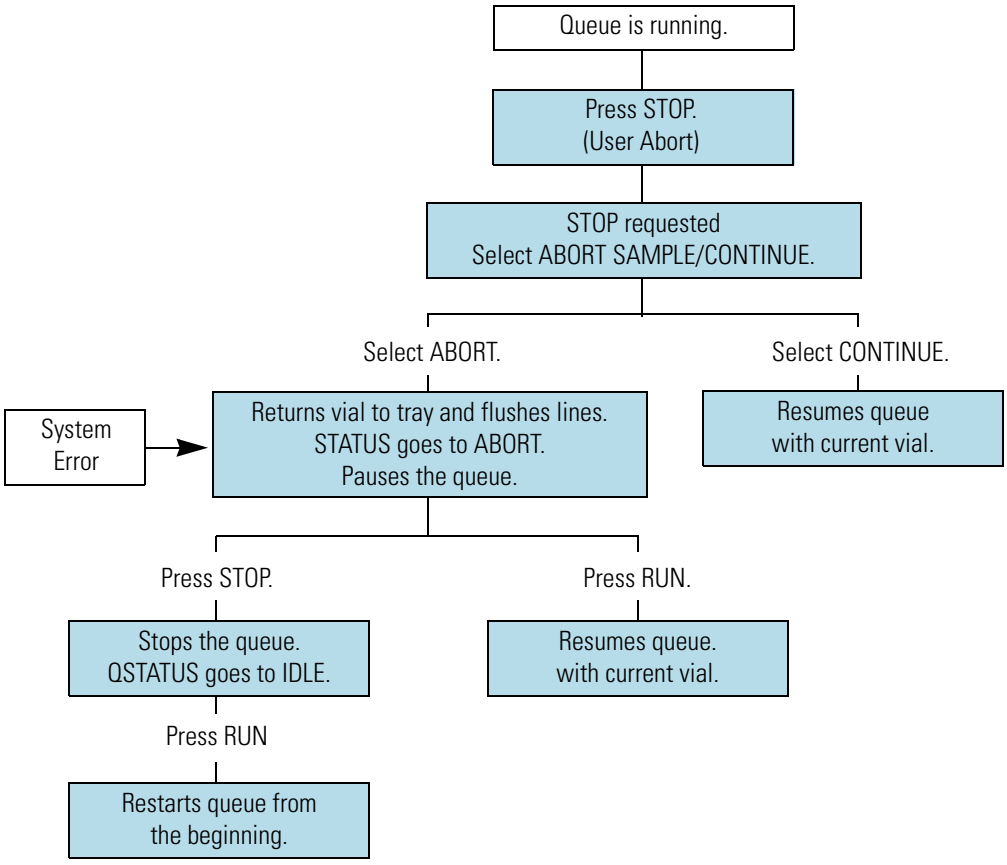
```
  Order   File Name      Set
  1       1:VITAMINS     P
-----------------------------------------------------------
  1       1:VITAMINS     1
  2       2:BARBITUA     2
  3       3:EXPER 1      3
```

Tip The door is equipped with a safety interlock that automatically moves the autosampler's arm to the home position to allow you to add samples to the tray during a run. Once the door is closed, the arm returns to its position and resumes the operation in progress before the door was opened. If necessary, however, you can disable the door interlock. Press the MENU key, and then select **OPTIONS > Ready Participation**. In the Door Interlock field, press the [+] and [–] keys to select **DISABLE**.

Stopping a Run

The STOP key has several functions depending on the instrument's status when you press the STOP key. See Figure 62 for an illustration of the Pause/Stop/Abort sequence.

Figure 62. Queue Stop/Abort sequence



If the queue is in RUN when you press the STOP key, the autosampler suspends its current operation until you select ABORT (see Figure 63) or CONTINUE (see Figure 64):

Figure 63. Abort prompt

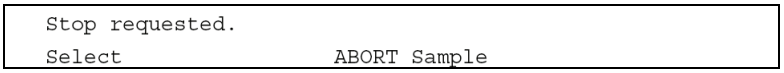


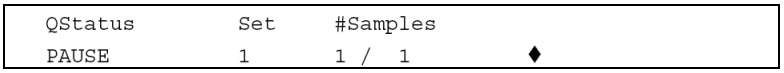
Figure 64. Continue prompt

If you select Continue (by pressing the [+] key), the autosampler returns the queue to the ACTIVE state and resumes the motor activity (as if you had not pressed the STOP key).

If you select Abort or if a system error occurs, the autosampler follows this sequence:

1. Cancels the processing of the current sample.
2. Returns the active vial to the vial tray.
3. Flushes the lines.
4. Puts the queue in the PAUSE state (Figure 65).

Figure 65. Queue Status line when the autosampler is in the PAUSE state



When the queue is paused, you can do one of the following:

- Press the RUN key to resume processing of the current vial.

  –or–

- Press the STOP key to stop the queue (the QStatus goes to IDLE), and then press the RUN key to restart processing from the beginning of the queue.

This STOP/Abort sequence gives you several opportunities to stop and resume or restart the run as needed.

Using the ABORT/CONTINUE selections and QPAUSE states, you can remedy problems that might adversely influence the processing or data received from the current or subsequent vials (depleted solvent supply, and so on) and resume the run without loss of previous data.

Using the ABORT selection and the STOP and RUN keys, you can stop the current run and remedy problems that might have influenced the processing or data received from all prior injections (incorrect file assigned to the set, incorrect solvent concentration, and so on). This second option minimizes loss of resources (and valuable time).

File Management

In addition to learning how to edit and load files from the Files menu, you can also copy and delete files, protect files from editing, and lock files to prevent changes to the run file during a run.

Saving Files

As you know, the autosampler automatically saves your file when you exit the Edit menu. If a power failure occurs while you are editing a file, you lose all current changes. However, if a power failure occurs after you exit the Edit menu, your changes are saved.

Copying Files

❖ To copy a file

1. Press the MENU key, and then select **FILES > Copy**.

   The Copy menu appears (see Figure 66).

   Figure 66. Copy menu

2. Enter the number of the file to be copied in the Copy File field.
3. Enter the number of the file you want to copy to in the To field.
4. Press the ENTER key.

   The **File Copied** message appears briefly and the application returns you to the Files menu.

Deleting Files

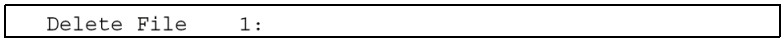
Deleting a file restores all parameters to their default values.

❖ To delete a file

1. Press the MENU key, and then select **FILES > Delete**.

   The Delete menu appears (see Figure 67).

   Figure 67. Delete menu

2. Enter the number of the file you want to delete and press the ENTER key.

   The **File Deleted** message appears briefly and the application returns you to the Files menu.

Protecting Files

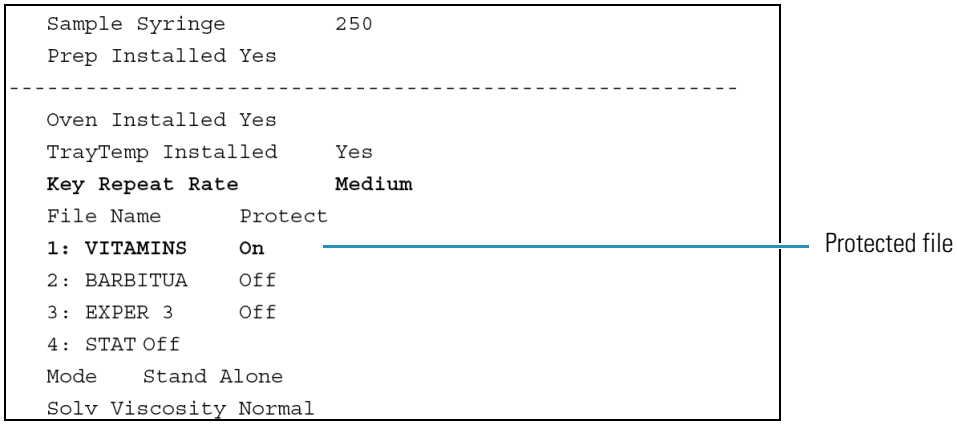
You can protect a file from being edited or deleted by using the file protection feature in the OPTIONS menu. Because this feature enables you to control the changes to an original run file, you can easily incorporate your autosampler files into your standard, good laboratory practice (GLP) protocols.

❖ To display the Configurations menu

Press the MENU key, and then select **OPTIONS > Configurations**.

The Configurations menu appears (see Figure 68).

Figure 68. Configurations menu



❖ To protect a file

1. Move the cursor down to the Protect field in the line that contains the file you want secured.

2. Press the [+] and [–] keys to turn the protect function to On.

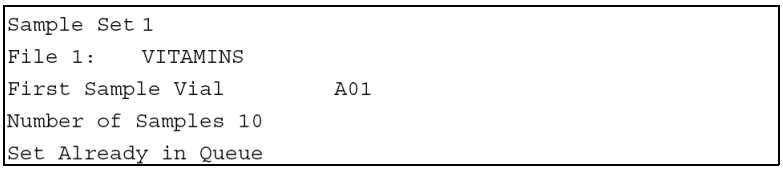
   For example, you cannot edit the Vitamins file shown in Figure 68 until you turn the protect function off. Attempting to edit a protected file displays the following message:

```
*File Protected*
No Editing Allowed
```

Changes to Real-Time Editing

If a file is protected, you cannot change the injection volume, number of injections per vial, or cycle time for the run in progress, and these lines are absent from the Samples menu as shown in Figure 69.

Figure 69. Samples menu for a protected file



Optimization

This section describes how to select the sample loop and syringe size to obtain maximum reproducibility when performing large-volume injections, how to adjust the draw speed for viscous samples, and how to adjust the needle height for sample vials containing either a limited sample volume or precipitates.

This section contains the following topics:

Injection Volume

The maximum injection volume depends on these conditions:

- The volume of the syringe currently installed as designated under OPTION > Configuration > Syringe Size
- The Injection Type selected under FILE > More > Injection Type
- The volume of the installed sample loop

By changing the sample syringe, the sample loop, or both, you can set up your autosampler to deliver injection volumes larger than can be delivered by the 250 µL syringe shipped with the instrument.

❖ To prepare your instrument for large-volume injections

1. Determine the correct sample loop size for the injection volume desired. See Table 17.
2. Use the injection volume and algorithms outlined below to determine the required syringe size.

   These algorithms define the amount of sample needed for your injection volume:

PushLoop	Injection Volume + 15 µL
Pull Loop	Injection Volume + 1.1 µL
Full Loop	Injection Volume × 1.33 + 70 µL

3. Change your sample loop and syringe. (For instructions, see "Changing the Sample Loop" on page 121.)
4. Enter the syringe size in the FILE > Configuration > Syringe Size field.
5. Enter the Injection Type in the OPTIONS > More > Injection Type field.
6. Enter your injection volume in the Injection menu.

Tip If you select the Full-loop type, the maximum value depends only on the loop size, assuming that the syringe size is larger than the loop size. (Loops come only in the sizes listed in Chapter 8, "Replaceable Parts.")

IMPORTANT For pull-loop and PushLoop injection, to ensure maximum reproducibility, do not inject more than half of the sample loop volume.

Table 17. Recommended injection size for selected sample loop sizes*

	Injection volume (in µL)		
	Injection type		
Sample loop size (µL)	**PushLoop**	**Pull**	**Full**
5	2.5	2.5	5
10	5.0	5	10
20	10	10	20
50	25	25	50
100	50	50	100
200	100	100	200
500	200	250	500
1000	200	500	1000

* These recommended volumes provide the best chromatographic performance. The type of system being used determines the maximum injection sizes.

The syringe size needed will be the total volume determined by these algorithms. For example, for a 500 µL full-loop injection (using a 1000 µL loop), the total volume (TV) needed is as follows:

TV = 500 µL × 1.33 + 70 µL

TV = 735 µL

You would then need a 1000 µL syringe, as there are no 735 µL syringes available. See Table 18.

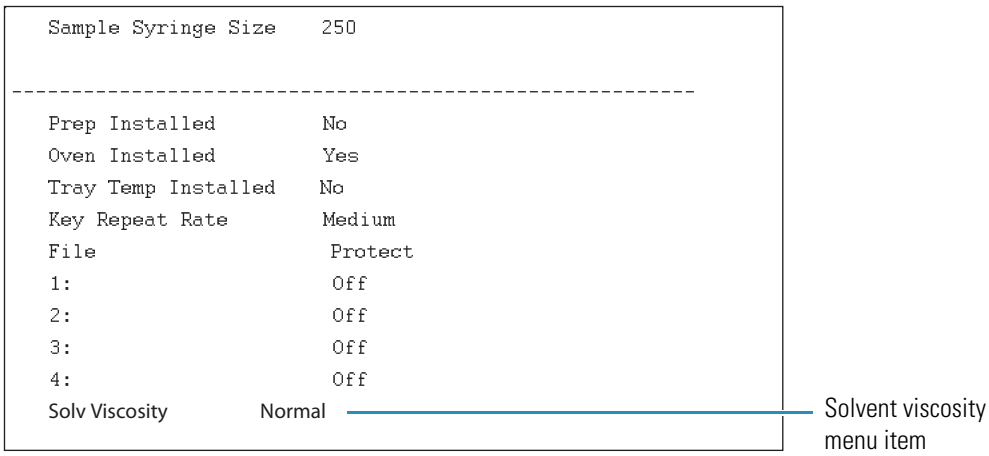
Table 18. Maximum injection size for syringe size

	Syringe size (in µL)				
Injection type	250	500	1000	2500	5000
Push Hi	200	200	200	200	200
Pull	212	461	956	1500	1500
Full	100	200	500	1000	1000

Solvent Viscosity

You can adjust the syringe speed (draw rate) for your solvent viscosity from the Solv Viscosity field in the Configurations menu (see Figure 70).

Figure 70. Configurations menu



Using the [+] and [–] keys, you can select from three different syringe speeds:

- Normal (fastest)
- Medium
- Viscous (slowest)

Use Normal to optimize your cycle time; Normal is optimal for most applications. With very viscous solvents or normal-phase (low viscosity) solvents, you might want to use the Viscous setting to slow down the syringe rate. This will prevent cavitation and nonreproducible injection volumes.

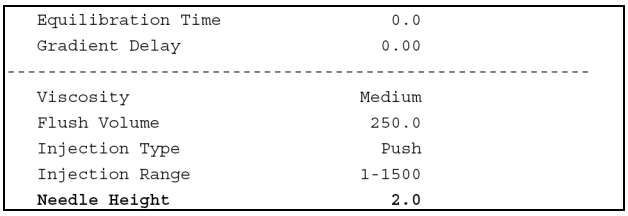
Needle Height

For some applications, such as liquid-liquid extractions and low-injection volumes, you might need to change or optimize the needle height. On the SpectraSYSTEM autosampler, the needle does not move. Instead, the bottom of the vial moves relative to the needle tip. The needle height is the approximate distance between the needle tip and the bottom of the vial (0 to 20 mm).

The default setting of 2 mm in the More menu is sufficient for most applications and accommodates a wide variety of vials (see Figure 71). However, cases where you have very limited sample volumes might require further optimization.

Note The thickness of the bottom of the vial varies considerably between vial types (1.8 mL vials, microvials, and vials with micro-inserts, and so on) and materials, such as glass or plastic.

Figure 71. Needle Height field in the More menu



IMPORTANT Operating the autosampler with a needle height that is different from the default height might bend the needle. Do not adjust the needle height without a spare needle available.

❖ To optimize the needle height for your sample vials

1. Fill a vial with 200 to 300 µL of water and place the vial in position A01.
2. Create an autosampler file that makes two 200 µL injections.
   a. In the Injection menu, set the Injection Volume field to **200**.
   b. Set Injections/Sample to **2**.
3. Run the file and observe the amount of fluid remaining in the vial. The fluid height indicates the position of the needle tip from the vial's bottom.
4. In the More menu, adjust the Needle Height value until the needle tip is located approximately 1 to 2 mm from the bottom of the vial.
5. Repeat steps 1 to 3 to confirm the needle height is 1 to 2 mm from the vial bottom.

Special Commands in the Commands Menu

The autosampler has several built-in commands for manually manipulating some of the autosampler's components (the arm, injection valve, and so on) outside of a run. These commands are useful during maintenance and troubleshooting procedures and are accessible from the Commands menu (see Figure 72).

Figure 72. Commands menu

These topics describe the commands available from the Commands menu:

- "Flush Sample Syringe" on this page
- "Initialize Hardware" on page 97
- "Injector" on page 97
- "Arm" on page 97

Flush Sample Syringe

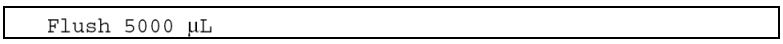
Use this command to manually flush the 250 µL sample syringe. You used this command when you primed your autosampler, but you can also use it for maintenance and troubleshooting.

❖ To access the flush sample syringe test

1. Fill your flush solvent bottle, if you have not already done so.
2. Press the MENU key, and then select **COMMANDS** (see Figure 72).
3. Select **Flush Sample Syringe**.

   The Flush command appears (see Figure 73).
4. Press the [+] and [–] keys to select the desired flush volume in microliters. Then press the ENTER key to initiate the flush operation.

   Figure 73. Flush command

The autosampler automatically flushes the sample syringe and flush lines with the specified volume of flush solvent and returns the syringe to its starting position. During the flush sequence a confirmation message is displayed.

Initialize Hardware

Use the Initialize Hardware command to manually control the autosampler's syringe and injection valve. This command brings the syringe to the initial position and removes any backlash.

Injector

Use the Injector command to manually switch the injection valve between the inject and fill positions. Selecting Injector displays the menu shown in Figure 74.

Figure 74. Injector command

```
Rotate Injection Valve INJECT
```

Pressing the [+] or [–] key and then pressing the ENTER key changes the injection valve's position.

Arm

The Arm menu contains a routine for returning the XYZ arm to its home position and a routine for aligning the arm. Figure 75 shows the Arm menu.

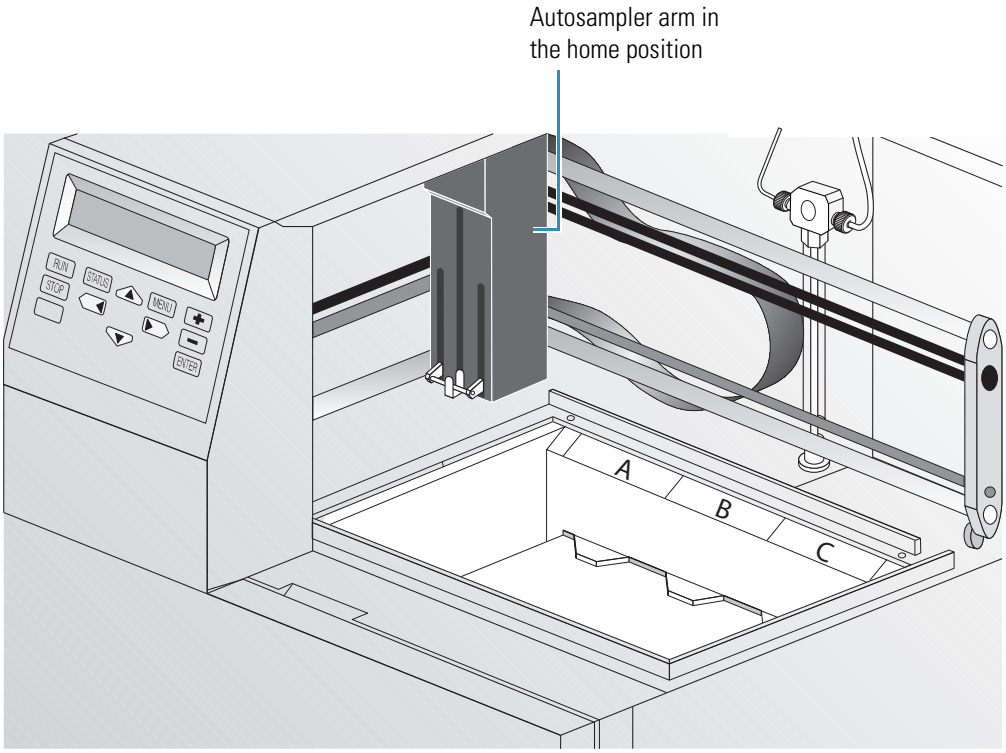
Figure 75. Arm menu

```
Home Arm
Align Arm
```

Home Arm

Pressing the ENTER key with the cursor in the Home Arm field moves the arm from its present position to its home location (the left side in the back-left corner of the tub). See Figure 76.

Figure 76. Autosampler arm in the home position



Align Arm

The Align Arm routine moves the arm to the vial position requested. It is used primarily by Thermo Fisher Scientific field service engineers to properly adjust the arm's x-y-z reference points. If you suspect that the arm's alignment is incorrect, call your Thermo Fisher Scientific service representative.

Figure 77. Align Arm command

```
Align Arm      A01
```

Diagnostic Procedures

This chapter describes the built-in diagnostics and how to determine the gradient delay volume of your LC system.

Contents

Gradient Delay

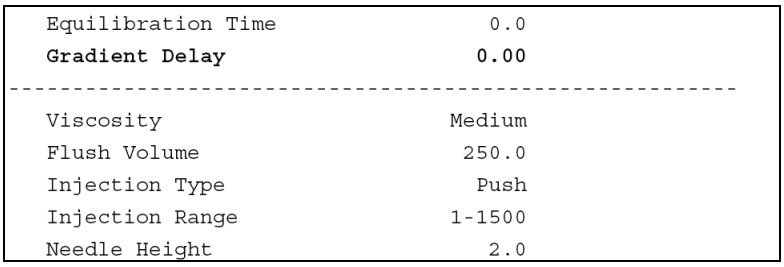
Gradient delay is a user-defined value that delays the injection of a sample. This delay is the amount of time required for a change in the mobile phase to travel from the gradient proportioning valve to the injection valve. The volume of mobile phase contained between the two valves is also referred to as the precolumn volume (PV).

Although not required for normal autosampler operation, Thermo Fisher Scientific recommends adding a gradient delay value to your Edit file when using large volume pre-filters or low flow rates (<0.5 mL/min).

❖ **To add the gradient delay time for your LC system to your Edit file**

1. From the main menu, select **FILES > Edit**.
2. Open your autosampler file.
3. Press the DOWN ARROW key (▼) until you reach the More menu, and then press Enter.
4. In the More menu, press the DOWN ARROW key (▼) until you reach the Gradient Delay field.

Figure 78. More menu



5. Press the [+] or [–] key to enter the gradient delay time for your LC system in minutes.

   The range is 0.00 to 99.99 minutes.

Calculating the Gradient Delay Time

❖ **To calculate the gradient delay time for your HPLC system**

1. Inject an unretained sample into your LC system with a column inline and using 100% A as the mobile phase (for example, MeOH).
2. Change to 100% B (for example, 0.1% acetone in MeOH) as fast as possible (0.1 minutes for SpectraSYSTEM pumps).
3. Subtract the retention time (RT) of the unretained peak from the retention time of the start of the baseline shift (T2 – T1).
4. Subtract the time required to switch solvents from 100% A to 100% B.

   The resulting time is the gradient delay time.

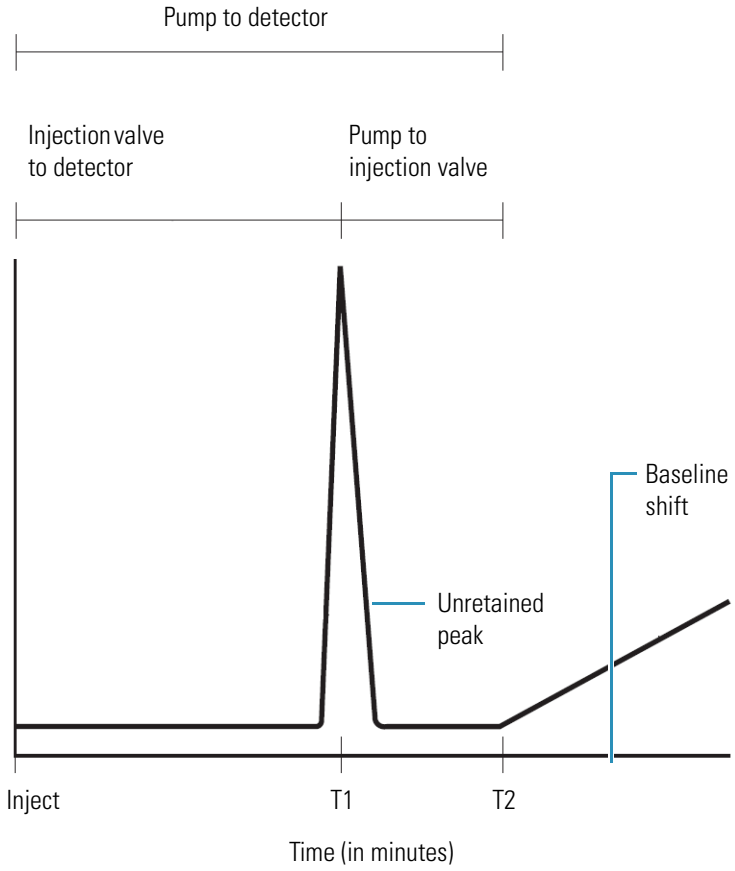
Gradient Delay Example

The following example illustrates how to determine the gradient delay volume for your LC system.

An unretained peak has a retention time of 3.0 min with a baseline shift at 4.5 min and a complete mobile-phase switch time of 0.1 min (see Figure 79). The gradient delay is calculated as follows:

$$\begin{aligned} \text{Gradient Delay} &= (T2 - T1) - 0.1 \text{ min} \\ &= (4.5 - 3.0) - 0.1 \\ &= 1.4 \text{ min} \end{aligned}$$

Figure 79. Chromatographic results for a gradient delay time experiment



Diagnostics in the Tests Menu

The SpectraSYSTEM autosampler has built-in diagnostic routines accessible from the Tests menu. Using these tests, you can access the operation of your autosampler. In addition to describing each of these tests, this section shows how the diagnostic tests are run and how to interpret the error screen information when a test fails.

All of the test screens are arranged similarly. You initiate a test by pressing the ENTER key. While a test is in progress, the name of the test is displayed along with three dots (Figure 80).

Figure 80. Test in-progress screen

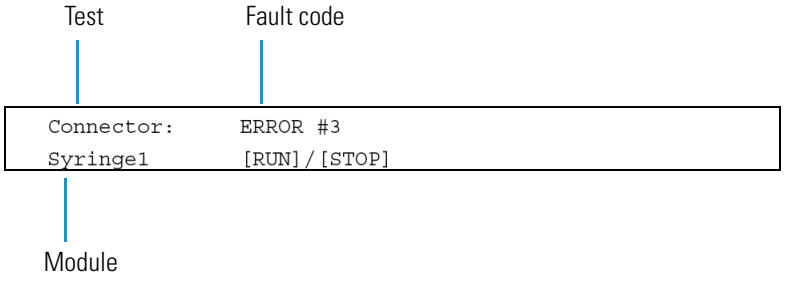
```
Test External Contacts:  ...
```

Interpreting the Results

When the test is complete, either a PASS or FAIL result is displayed. When a test fails, an error screen similar to that shown in Figure 81 appears with the following information (described in Table 19):

- Test identification
- Fault code (diagnostic error)
- Module identification
- [RUN] / [STOP]

Figure 81. Error screen for a diagnostic test



Note The error number in this screen is just an example to show you the screen layout.

Table 19. Error screen fields from top left

Field	Description
Test identification	The name of the test being run.
Fault code	The error number associated with the specific failed result. To receive further assistance in diagnosing and solving the problem, document and report the fault code to your service representative.
Module identification	Displays the specific autosampler board or assembly where the problem occurred.
[RUN / STOP]	Prompts you to press the RUN key to repeat the test or to press the STOP key to exit the current test and return to the Tests menu.

Test Descriptions

All diagnostics and built-in assessment routines are accessible from the Tests menu (Figure 82). This topic describes the items in the Tests menu.

Figure 82. Tests menu

Software Version

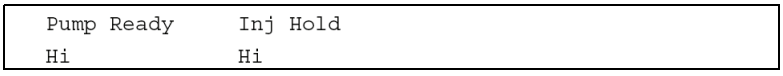
Selecting this field displays the current software version number, *x.yy.zz*.

Display Input States

This menu (Figure 83) shows the current states of the TTL-input device signals (Hi or Lo). You can use this information to check the level of the Pump Ready and Injection Hold input lines from other LC system modules. You can also diagnose situations where the autosampler is waiting for ready or waiting to inject. Make sure the Input Polarity is set to the proper level for each of these signals. Pressing the UP or DOWN ARROW key (▲ or ▼) returns you to the Display Input States field in the Test menu if you want to repeat the test.

Tip You can change the active polarity of a specific input signal from the OPTIONS > Input Polarity menu.

Figure 83. Display Inputs States menu

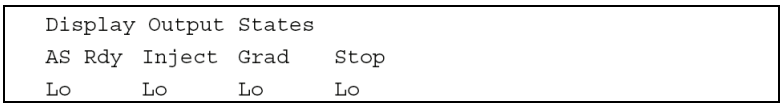


Display Output States

This menu shows the current states of the TTL-output device signals (Hi or Lo). Use this information when hardwiring your autosampler to other LC system modules. Pressing the UP or DOWN ARROW key (▲ or ▼) returns you to the Display Output States field in the Test menu if you want to repeat the test.

Tip You can change the active polarity of a specific input signal from the OPTIONS > Input Polarity menu. This menu shows the states of the TTL output device signals (Hi or Lo) as shown in Figure 84.

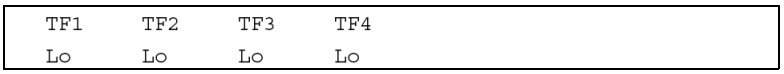
Figure 84. Display Output States menu



Display Timed Events

This menu (Figure 85) displays the current states of the timed events outputs. Use this information when hardwiring your autosampler to other LC system modules. For more information, see "General Chromatography Troubleshooting" on page 129.

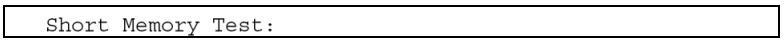
Figure 85. Display Timed Events menu



RAM Test

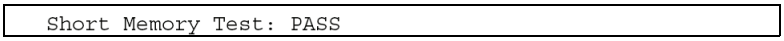
This test (Figure 86) writes to and then reads the contents of the random access memory. If the write information is the same as the read information, the test is successful. You might want to run this test once a month to verify proper software operation.

Figure 86. RAM Test menu



If the test is successful, the autosampler returns a PASS result (Figure 87). If unsuccessful, the autosampler returns a FAIL result and displays an error message. Document the test results and error message, and call your Thermo Fisher Scientific service representative.

Figure 87. Successful RAM Test



Press the STOP key to return to the Tests menu.

ROM Test

This test evaluates check-sums on the contents of the instrument's ROM and compares the results to known values.

If the test is successful, the autosampler returns a PASS result. If unsuccessful, the autosampler displays the number of the failed chip or chips, along with the regular error screen information (Figure 88). If the test fails, document the test results and call your Thermo Fisher Scientific service representative.

Figure 88. Failed ROM Test

Hardware Test

The hardware test is the most important autosampler diagnostic. It checks all of the cable connections, sensors, motors, circuitry, and attached components.

Before running this test, be sure that the XYZ arm is in the home position (at the left back of the sample compartment), or the AS3000 autosampler might report false errors. You can use the Home Arm command in the Commands menu (see "Special Commands in the Commands Menu" on page 96) if the arm is not in the home position.

If the Hardware Test fails, the screen displays the test identification, fault code, applicable module or connection, and [RUN] / STOP] fields. For example, in Figure 89, the message J10/J40/J45 indicates that further troubleshooting is required. Document the screen information and report it to your Thermo Fisher Scientific service representative when you call for assistance.

Figure 89. Example error screen

```
Connector:      ERROR #3
J10/J40/J45     [RUN]/[STOP]
```

Pressing the STOP key returns you to the Tests menu.

Most of the failed Hardware Test results require that you contact your service representative for assistance. However, two of the messages indicate problems that you can likely resolve without a service call.

- Opt Config

  This message indicates an incorrect configuration. Check the Configurations menu (under OPTIONS) and verify that the options field entries match your installed options.

- Arm,*

  This message indicates that the XYZ arm was not in its home position when the Hardware Test was initiated. Select **COMMANDS > Arm > Home arm**.

Test External Contacts

This test activates all of the external contacts and tests for proper activation. Do not run the test while the external contacts are wired to other equipment, or the test will report false errors. Before running this test, disconnect the 12-pin connector block from the back of the instrument. After the test is complete, plug the connector back in to resume normal instrument operation.

Pressing the ENTER key with the cursor in the Test External Contacts field displays a warning prompt (Figure 90).

Figure 90. External Devices menu

```
Disconnect all external contacts      CONTINUE
```

From this menu, you can use the [+] and [–] keys to select **CONTINUE** or **ABORT**. Selecting Continue starts the test.

Selecting ABORT discontinues the test and returns you to the Test External Contacts field of the Tests menu. If ABORT is displayed and you want to resume the test, press the LEFT or RIGHT ARROW key. The display changes from ABORT to CONTINUE.

Test COMM Port

Use this test to assess the operation of the communications port on the autosampler's back panel. This test sends a signal to the communications port and expects a signal in return. A successful result is reported as a PASS; an unsuccessful result is indicated by the regular error screen.

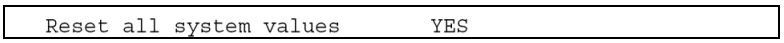
To perform this test, you must connect a loop-back connector to the communications port.

Tip If you want to perform this test, contact your Thermo Fisher Scientific field service engineer for the procedure and the necessary parts information.

Initialize EEPROM

The Electronically Erasable Programmable Read Only Memory (EEPROM) stores all files, user-set variable values, and system configuration information when the power is turned off. Reinitializing the EEPROM restores the default values to all files and erases the system configuration information. Pressing the ENTER key in this field displays the message shown in Figure 91.

Figure 91. Initialize EEPROM menu



If you want to exit this routine before running it, you can do any of the following:

- Press the [+] and [–] keys to select **No**.
- Press the MENU key to open the main menu.
- Press the STATUS key to open the Status Screen.

Maintenance

This chapter contains the maintenance schedule and procedures for your autosampler.

Contents

Specific Hazards

For your own safety and to protect the autosampler from damage, follow the safety precautions in this manual when performing maintenance on the autosampler.

CAUTION Alerts you to situations that could result in personal injury and tells you how to avoid them.

CAUTION High Voltage. Alerts you to the presence of high voltage and to the potential injury that could occur from electrical shock if you came into contact with a specific instrument area or component. Also indicates how to avoid contact with the high-voltage areas in your instrument.

CAUTION Hot Surface. Alerts you to potential injury that could occur from contact with a heated surface or area on or in an instrument. Also indicates how to avoid contact with the heated surfaces in your instrument.

Maintenance Schedule

Your autosampler requires only a few simple maintenance procedures to keep it in optimal working condition. Table 20 contains the maintenance schedule for the autosampler. Ensure that only a Thermo Fisher Scientific service representative or other qualified person performs the yearly maintenance tasks.

CAUTION To avoid electrical shock, do not remove the top cover or remove parts beyond the descriptions in the operation portions or in the maintenance topics of this manual.

Table 20. Maintenance schedule

Frequency	Task	Performed by
Daily	Empty flush-solvent tray.	User
Monthly	Inspect the sample needle module.	User
	Check the solvent tubing and connections for leaks.	User
	Clean the drop catch.	User
Semi-annually	Replace the flush-solvent inlet filter.	User
	Replace the needle assembly.	User
Annually or as necessary	Replace the injection valve's rotor seal.	User
	Clean and lubricate the syringe drive mechanism.	Service
	Clean and lubricate the XYZ arm.	Service
	Verify the XYZ arm alignment.	Service

IMPORTANT You are responsible for maintenance of the autosampler. Routine maintenance is not provided under warranty; however, planned maintenance contracts are generally available. If you are interested in purchasing a planned maintenance contract, contact your local service representative.

This chapter contains recommended maintenance intervals based on operating the autosampler under typical conditions. Operating the autosampler under certain harsh conditions (corrosive solvents, extreme temperatures, and so on) might require more frequent maintenance than indicated in this chapter.

Maintenance Procedures

This section contains the following maintenance procedures:

Emptying the Flush-Solvent Tray

For normal operation, you might need to empty the tray at least once or twice a day.

❖ **To empty the flush-solvent tray**

1. Be sure the autosampler is in IDLE status.
2. Carefully slide the tray out without spilling the collected solvent.
3. Empty the tray, rinse it off with water, and slide it back into place under the elbowed, barbed piece of black tubing.

CAUTION Wear skin and eye protection and dispose of hazardous waste according to local, state, and federal regulations.

IMPORTANT Be sure that the level of the liquid in the waste container is below the level of the instrument, or waste will collect in the lines and can backflush into the instrument.

Replacing the Rotor Seal

To replace the rotor seal, follow these procedures in order:

1. Removing the Injection Valve
2. Disassembling the Injection Valve
3. Cleaning the Stator
4. Installing the Rotor Seal
5. Reinstalling the Injection Valve

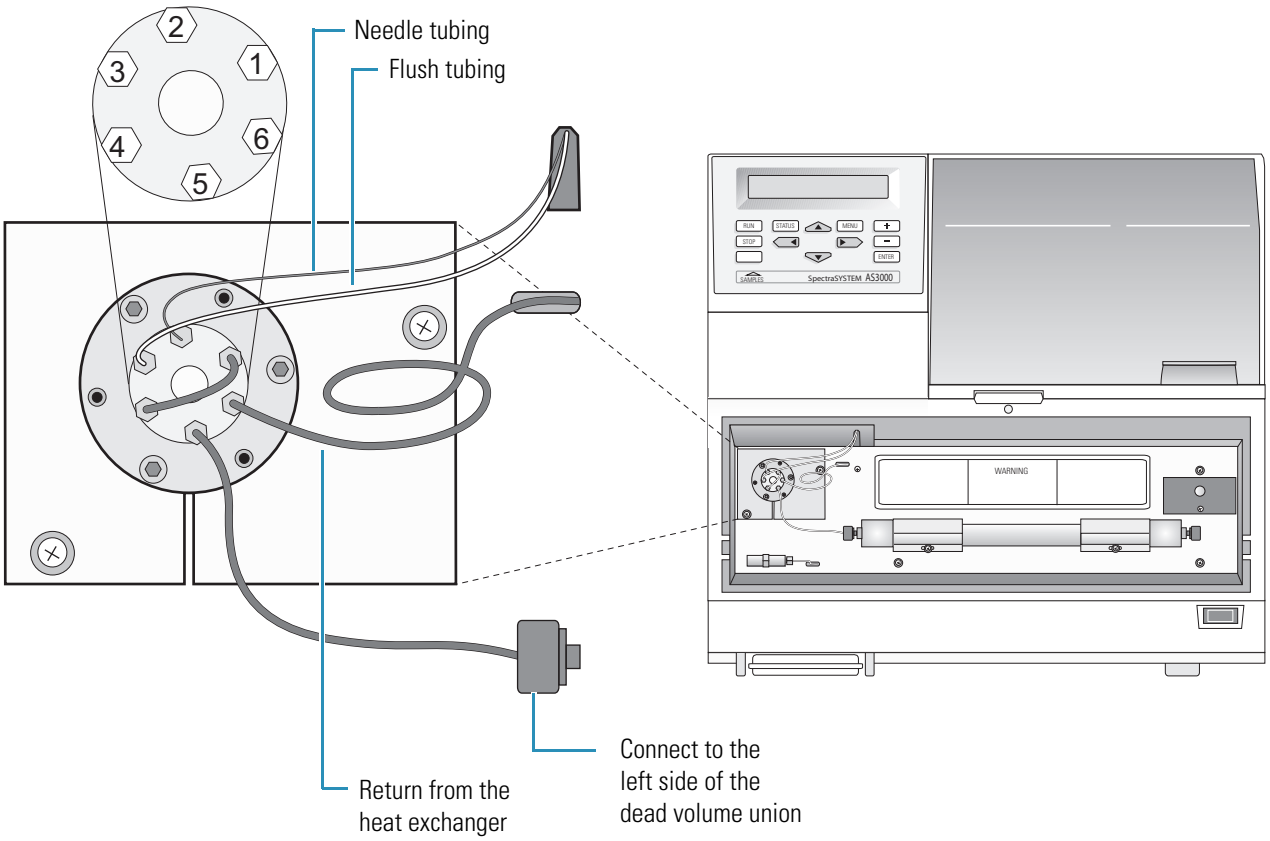
Removing the Injection Valve

If you are only replacing the rotor seal, you do not have to remove the injection valve from the instrument; however, it is easier if you do. If you want to replace the rotor seal with the valve installed, proceed to the section entitled, "Disassembling the Injection Valve" on page 111.

❖ To remove the injection valve from the autosampler

1. Using a 1/4 in. open-end wrench, disconnect the tubing from ports 2, 3, 5, and 6 of the injection valve (see Figure 92).
2. Remove the sample loop.
3. If your autosampler has a column oven, loosen and remove the two Phillips-head screws that secure the injection valve collar to the front of the oven. Then remove the collar.
4. Remove the two Phillips screws that secure the valve mounting plate.
5. Carefully pull the injection valve out of the instrument.

Figure 92. Injection valve tubing connections



Disassembling the Injection Valve

To replace the rotor seal, disassemble the injection valve on a clean benchtop.

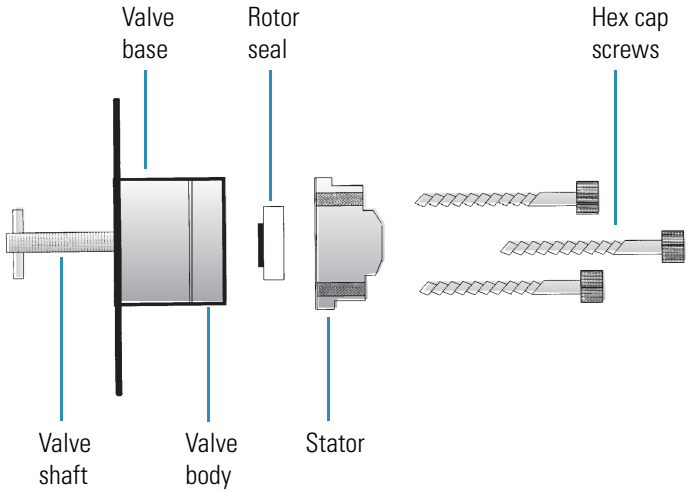
IMPORTANT Do this repair in a clean environment. Component cleanliness affects the useful life of your injection valve.

IMPORTANT Do not activate the valve mechanism with the injection valve removed from the instrument or risk damaging the valve mechanism.

❖ To disassemble the injection valve

1. Remove the injection valve from the autosampler (see "Removing the Injection Valve" on page 110).
2. Using an Allen wrench, remove the 9/64 in. hex cap screws from the top of the valve (Figure 93).
3. Carefully lift the stator from the valve body and set it onto a clean surface.

Figure 93. Injection valve components

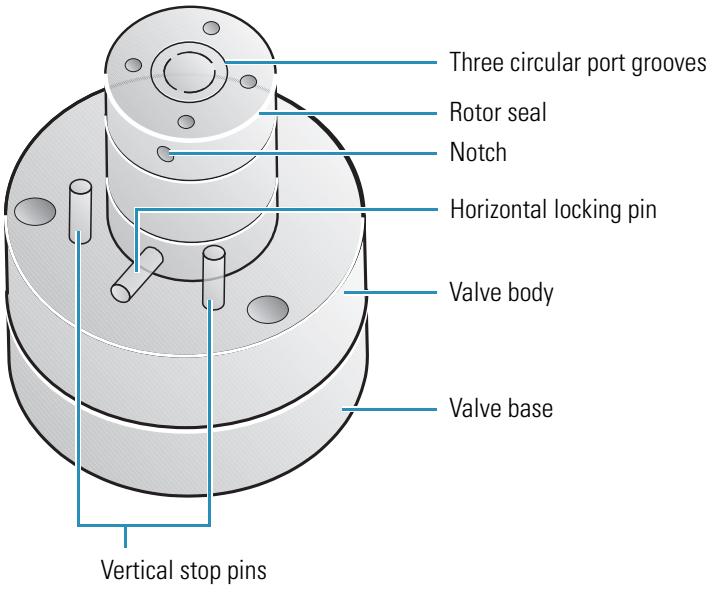


Note Be careful not to drop the two rotation stop pins that protrude from the valve base (Figure 94).

4. Remove the valve body.
5. Before removing the worn rotor seal, note these details on the rotor seal (Figure 94):
   - The three circular port grooves on one side of the rotor (the other side is smooth)
   - The small locating notch on the rotor's rim (not the V stamped on the seal)

   Figure 94 shows the injection valve with the valve body removed and the rotor seal exposed. The valve's mounting bracket is not shown.

Figure 94. Injection valve with valve body removed and rotor seal exposed



6. Remove the worn rotor seal. You might need a thin, flat-bladed tool to remove the seal.

Cleaning the Stator

Determine whether the stator requires cleaning by inspecting it.

❖ To clean the stator

1. If it is dirty or greasy, swab the stator with HPLC-grade methanol. If more stringent cleaning is required, use a sonicator.
2. Inspect the remaining valve components for contamination. Clean as necessary.

IMPORTANT If the stator is scratched, replace it. Scratches can damage the rotor seal and cause valve leaks.

Installing the Rotor Seal

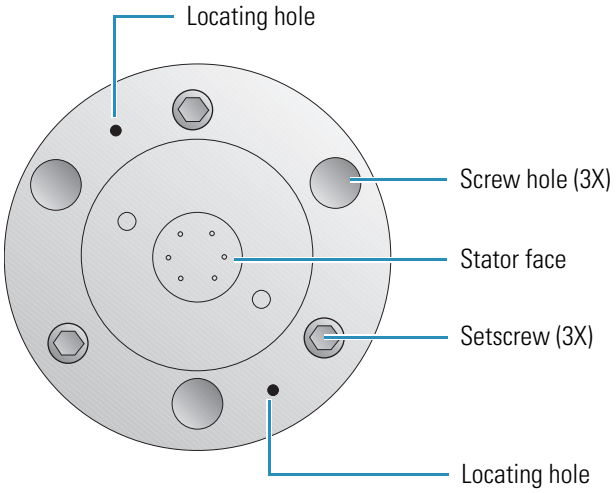
❖ To install the rotor seal

1. Install the new rotor seal in exactly the same orientation as the old one. Be sure that the rim notch is in line with the horizontal locating pin and that the circular port grooves are visible.
2. Install the valve body.

3. Install the stator onto the valve body carefully, observing that the two locating pins in the valve body (Figure 94) line up with the two holes on the bottom side of the stator assembly (Figure 95).

IMPORTANT Be sure to hold the valve shaft so that the horizontal locating pin touches the left stop pin as you reassemble the valve. This puts the valve into the INJECT position (the same position as when you removed the valve from the instrument). If the pins are not in the correct orientation, the valve shaft might not be in the proper orientation for reinsertion into the instrument.

Figure 95. Bottom of the stator assembly



IMPORTANT These locating pins orient the stator assembly in one of two positions, but only one position aligns the mounting holes in the valve body with the screw holes in the stator.

4. Insert the three cap screws so that each one just contacts the stator.

IMPORTANT To maintain the necessary pressure for proper valve operation and to avoid valve binding, look for a uniform gap between the stator and the valve body. Do not over-tighten the valve.

5. Tighten each cap screw an equal amount until all setscrews are in equal contact with the valve body.

Reinstalling the Injection Valve

If you removed the injection valve from the autosampler to replace the rotor seal, reinstall the injection valve.

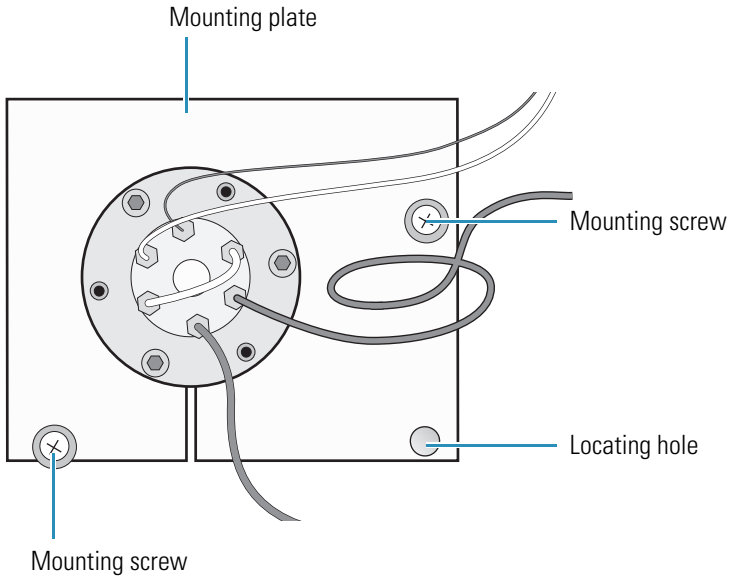
❖ To install the injection valve

1. Align the valve shaft with the port for the injection valve.
2. Secure the valve mounting plate with the two Phillips screws.

   Figure 96 shows the correct orientation for the valve mounting plate. The locating hole is below and to the right of the injection valve.

   Note When reinstalling the mounting plate and valve assembly, be sure to orient the bracket with the corner that has the locating hole in the lower right as shown in Figure 96. Be sure that you can see the pin through the locating hole.

   Figure 96. Valve mounting plate in the correct orientation



3. Reconnect the sample loop to ports 1 and 4 of the injection valve.
4. Reconnect the tubing to ports 2, 3, 5, and 6 of the injection valve. Connect the needle tubing to port 2, the flush tubing to port 3, the dead volume union to port 5, and the return tubing from the heat exchanger to port 6. Using a 1/4 in. open-end wrench, tighten the fittings.

Replacing the Flush Solvent Inlet Filter

Properly filtering your flush solvent extends the life of your injection valve and column.

❖ **To replace the inlet filter**

1. Be sure the autosampler is in the IDLE mode.
2. Remove the flush-solvent bottle cap and lift out the inlet tubing.

   IMPORTANT Be sure to follow all of the safety precautions for each solvent as prescribed by the manufacturer's MSDS.

3. Put on a new filter cartridge and put the line back into the bottle. Tighten the solvent cap.
4. Flush the inlet filter and lines with an appropriate volume of solvent (~5 µL).

Maintaining the Sample Needle Module

To remove or clean the sample needle module and flush the drop catch, follow these procedures:

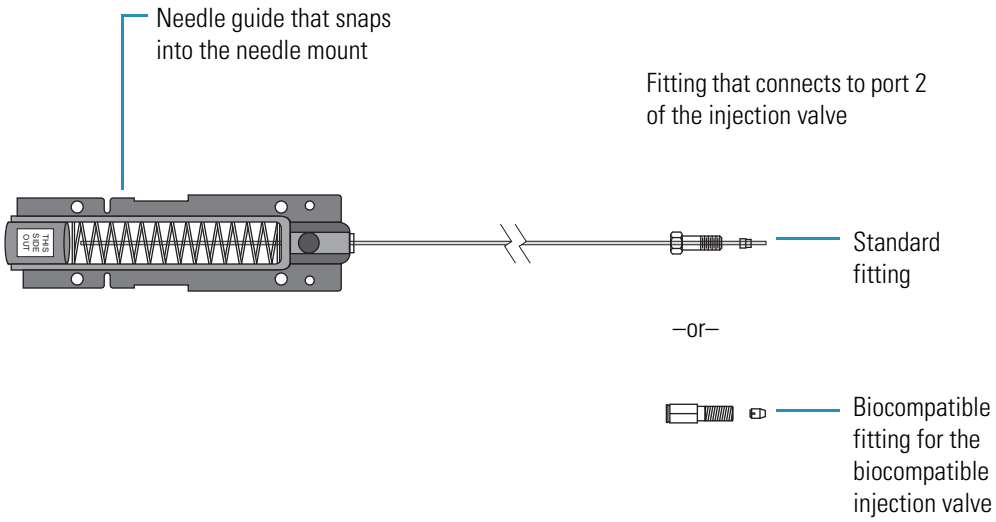
- "Removing or Cleaning the Sample Needle Module" on this page
- "Flushing the Drop Catch" on page 118

Removing or Cleaning the Sample Needle Module

The sample needle module resides in the sample tower and is responsible for piercing the vial septum and removing sample from the vial. The needle guide end of the module snaps out of the needle mount. The tubing portion of the assembly is routed through a hole in the column oven compartment and is connected to port 2 of the injection valve.

Figure 97 shows the sample needle module. Thermo Fisher Scientific supplies two versions of this module: a standard version and a biocompatible version. If your autosampler has a biocompatible injection valve, order the biocompatible version.

Figure 97. Sample needle module

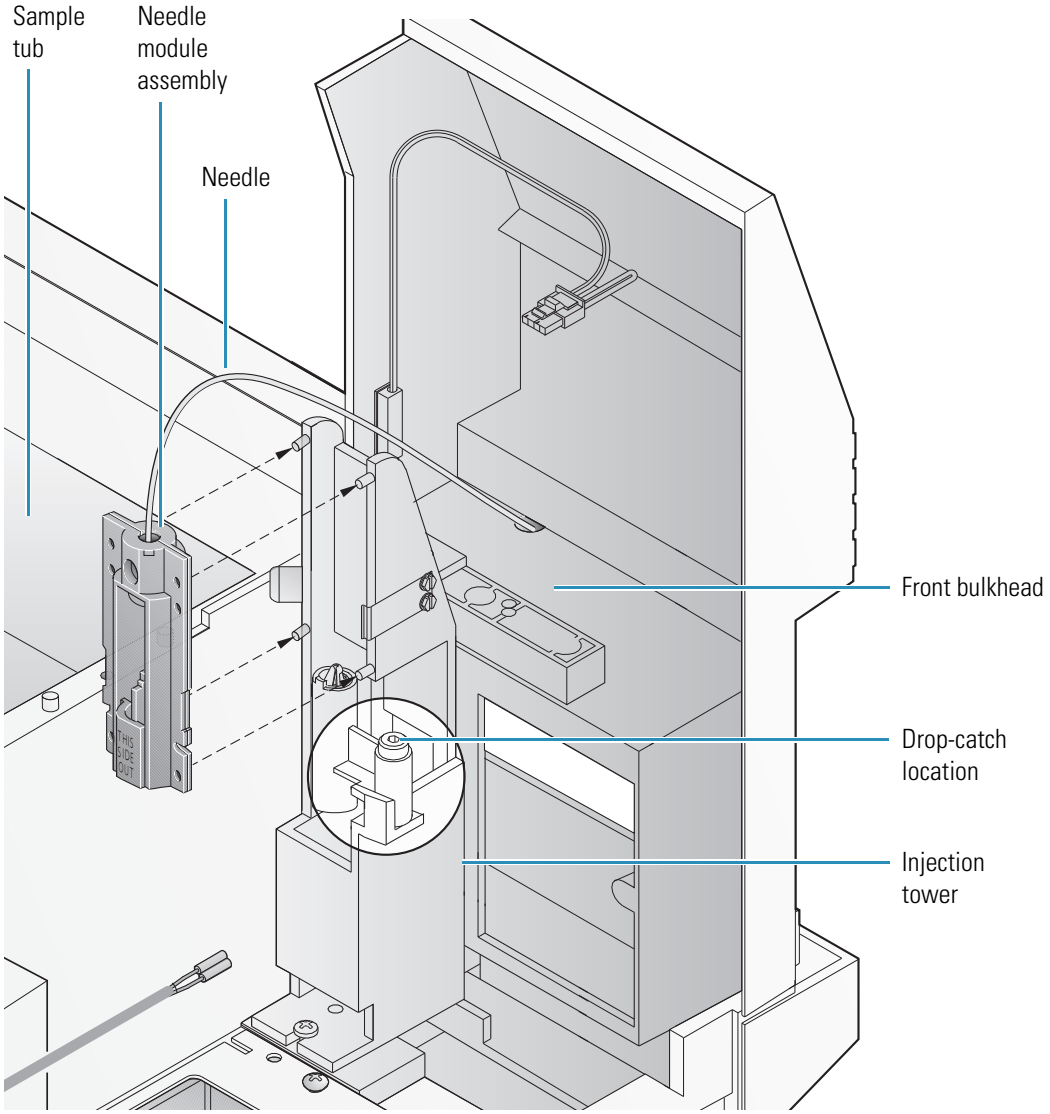


❖ To remove the sample needle module

1. Using a 1/4 inch open-end wrench, remove the tubing fitting from port 2 of the injection valve.
2. Carefully (to avoid damage to the tubing) release the sample needle module from the tower by grasping the module edges and pulling toward the back of the autosampler.
3. Pass the needle fitting through the autosampler's front bulkhead and remove the needle module from the autosampler.

Tip If you are replacing (not inspecting) the needle, you might want to cut the used needle to make it easier to pass the fitting through the bulkhead.

Figure 98. Sample needle module pulled away from the needle mount



❖ To clean the sample needle module

1. Inspect the needle module.
2. Rinse in water or sonicate if contamination is present.
3. If internal plugging is suspected, pass a cleaning wire through the needle and flush the needle with high flow from an LC pump.

IMPORTANT The sample needle module is glued during manufacture and cannot be disassembled.

❖ **To replace the sample needle module**

1. Remove the new module from the replacement kit.
2. Pass the valve end of the needle through the hole on the underside of the column compartment.
3. Snap the needle assembly into the mounting brackets on the backside of the sample tower (Figure 98).
4. Place the tube fitting and ferrule onto the needle and install it into port 2 of the injection valve (Figure 92).

Flushing the Drop Catch

The drop catch (Figure 98) removes the last drop of sample from the end of the needle after injection. Flush it monthly.

❖ **To flush the drop catch**

1. Remove the needle module assembly as described on page 116, but leave the drop catch installed.
2. Flush the drop catch with water, an organic solvent, or an acid or base (as appropriate for the sample), and once more with water. Then dry it with a clean tissue.

Replacing the Syringe

This topic describes how to replace the sample (250 µL) syringe (see Figure 99). You can replace the 250 µL sample syringe without removing the syringe drive assembly, but you must move the syringe drive about two inches out by interrupting a flush cycle.

Figure 99. Syringe

❖ To remove the sample syringe

1. From the main menu, select **Commands > Arm > Align Arm**. Press ENTER.

   The autosampler arm moves to position A01at the front left of the tray compartment.

2. From the main menu, select **Commands > Flush Sample Syringe**.

3. When the plunger is lowered, turn off the power.

4. Hold the silver base of the plunger to prevent it from turning, and loosen the knurled syringe set screw under the syringe's plunger.

   Tip You might need to use a needle nose pliers to loosen the set screw.

   Note The syringe set screw is a captive fitting and will not fall into the instrument's chassis.

5. To loosen the top of the syringe, hold the metal tip on the top of the syringe barrel, unscrew the used syringe barrel, and lift it out of the instrument.

   IMPORTANT Do not try to loosen the syringe by holding the barrel. The syringe could break between the glass barrel and the metal tip.

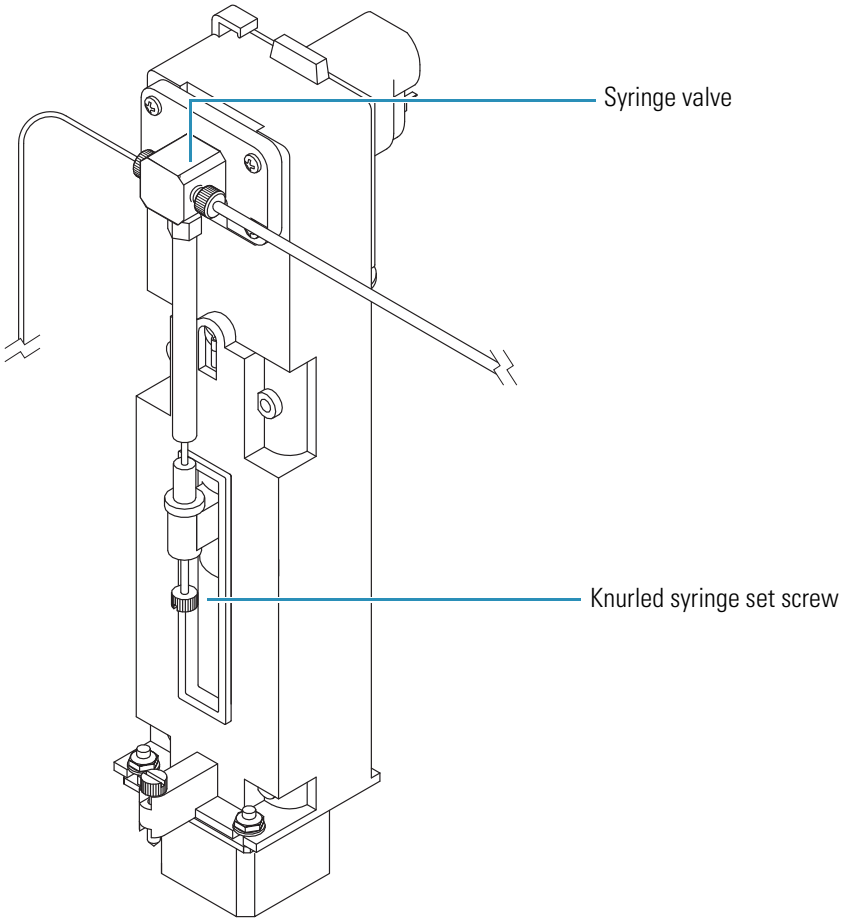
   Tip Push up on the syringe plunger while lifting it out of place.

   Tip Install the syringe in its fully expelled position to prevent an air bubble in the syringe barrel.

❖ To install the new syringe

1. Slide the syringe onto the syringe drive assembly (see Figure 100).

   Figure 100. Syringe drive assembly with syringe



2. Screw the syringe nut into the syringe valve.
3. Screw the syringe drive assembly nut into the silver base of the syringe plunger.
4. Turn the autosampler on.

   Because the arm is not in the home position, an error condition occurs (#140) as the autosampler goes through its initialization process.

5. Press RUN to clear the error.

Tip If you change the syringe size, do one of the following:

- If you are using the autosampler in the Stand Alone mode, change the syringe size in the **Options > Configurations > Sample Syringe** field.
- If you are using a chromatography data system to control the autosampler, enter the syringe size when specifying the configuration settings for the autosampler.

Changing the Sample Loop

A number of different size sample loops are available for the SpectraSYSTEM autosampler. For the list of spare parts and consumables, see Chapter 8, "Replaceable Parts."

❖ To change the sample loop

1. Be sure that the autosampler is in the IDLE mode.
2. Using a open-end wrench, loosen the fittings at ports 1 and 4, and pull out the two ends of the sample loop.
3. Insert the ends of the new sample loop in the same orientation as the old one and tighten the fittings.

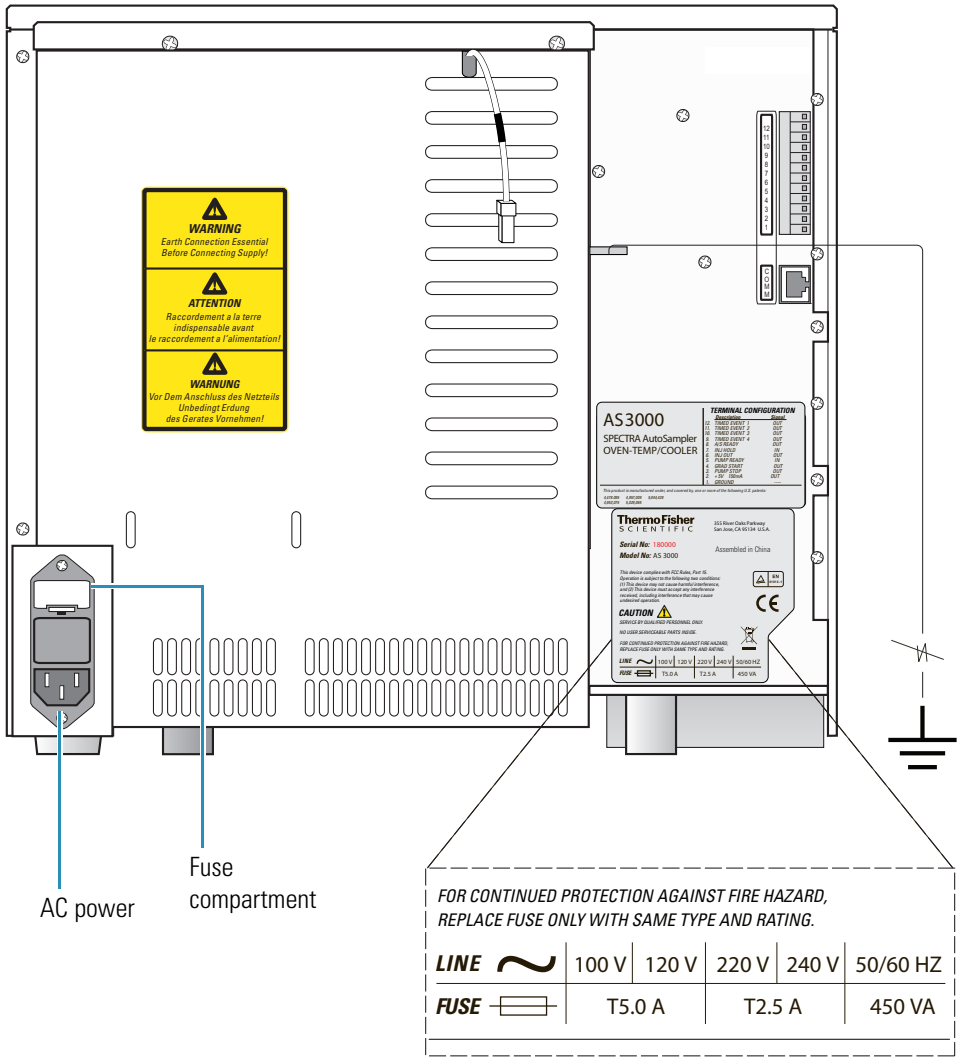
Tip If you are using the autosampler in the Stand Alone mode, enter the nominal sample loop size in the **File > Injections > Loop Size** field when making full-loop injections.

If you are using a chromatography data system to control the autosampler, enter the sample loop size when specifying the configuration settings for the autosampler.

Replacing the Fuses

Instrument power is supplied by two time-lag fuses housed in the fuse compartment on the lower left of the instrument's back panel (Figure 101).

Figure 101. Autosampler's back panel



CAUTION Disconnect the power cord before you replace fuses.

❖ To replace the fuses

1. Turn off the autosampler by pressing the on/off button on the lower right corner just below the front panel.
2. Unplug the power cord from the AC power receptacle on the autosampler's back panel.

3. Use a flat blade to press down on the plastic tab at the top edge of the fuse compartment and pull out the fuse holder.
4. Pull both fuses out of the holder and discard. Place the new fuses into the holder with the metal end visible.
5. Snap the fuse holder back into place.

Automated Shutdown in the Stand Alone Mode

If you are controlling the autosampler in the Stand Alone mode, you can set it to shut down the system during periods of disuse, for example, after completing an overnight or over-the-weekend run. The shutdown feature can turn off the autosampler's controlled temperature zones, flush the injection system, and send a signal to the pump to stop the pump flow. Enter the shutdown parameters in the OPTIONS > Shutdown menu (see Figure 102).

Figure 102. Options menu

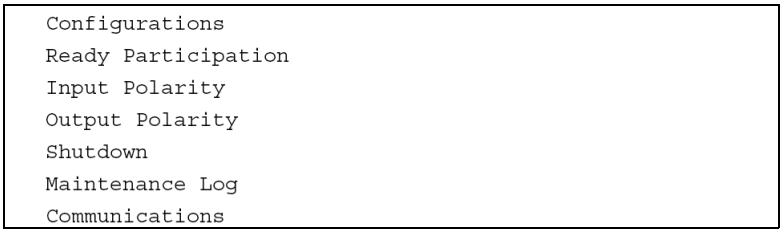


Figure 103 illustrates the autosampler's Shutdown menu. Table 21 describes the parameters.

Figure 103. Shutdown menu

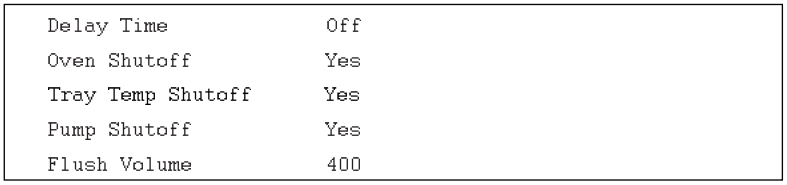


Table 21. Shutdown menu parameters (Sheet 1 of 2)

Parameter	Description
Delay Time	Specifies the desired amount of time that the autosampler is to wait before initiating the shutdown procedure. When you select Off, the autosampler performs the shutdown procedure as soon as the autosampler state returns to IDLE. Selections: Off, 5, 10, 20, 30, 45, 60, 90, 120, 240, and 480 minutes
Oven Shutoff	Selecting Yes causes the autosampler to automatically turn off the oven after the Shutoff Delay time has elapsed.

Table 21. Shutdown menu parameters (Sheet 2 of 2)

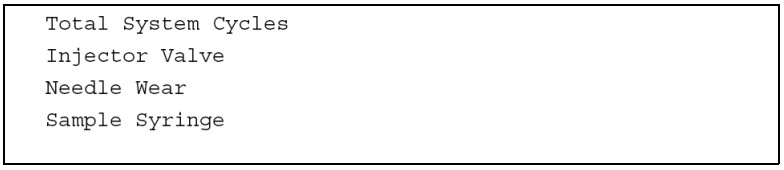
Parameter	Description
	Note If you are using the Shutdown function with methods that call for heating and cooling, be sure to allow a long enough delay time (10 or more minutes) for thermal equilibrium to occur.
Pump Shutoff	Selecting Yes causes the autosampler to automatically activate the Pump Stop output after the Delay Time has elapsed. Turning off the pump retains your solvent supply and prevents air from being pumped into the lines should the solvent reservoirs become depleted.
Flush Volume	Flushes the injection system with the specified volume. Range: 200 to 5000 µL Default: 400 µL

Maintenance Log

The Maintenance Log menu (see Figure 104), accessible from the OPTIONS menu, monitors the autosampler maintenance status and counts, and displays the number of activations for each of the routine maintenance parts listed in the menu.

Note If you are controlling the autosampler in the SpectraNet mode, set up and monitor the maintenance log from your chromatography data system.

Figure 104. Maintenance Log menu



For information on the Maintenance Log menu items, see these topics:

- "Instrument Maintenance Status" on page 125
- "Total System Cycles Menu" on page 125
- "Injector Valve Menu" on page 125
- "Needle Wear Menu" on page 127
- "Sample Syringe Menu" on page 127

Instrument Maintenance Status

The Maintenance Log menu provides a convenient way to keep track of autosampler maintenance. When you first access the Maintenance Log menu from the OPTIONS menu, the Maintenance Required confirmation message appears if instrument maintenance is required. This message is displayed if any of the #Cycles field values in any of the Maintenance Log menus is greater than the number of cycles entered in the Due fields. See the other topics in this section for more information on these fields.

If maintenance is not required, no message appears. Pressing the DOWN ARROW key (▼) clears the confirmation message and returns you to the Maintenance Log menu.

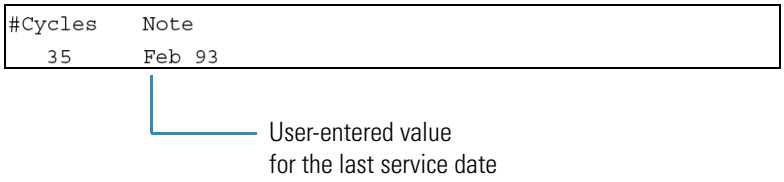
Total System Cycles Menu

The Total System Cycles menu (see Figure 105) displays the total number of instrument cycles in the #Cycles field. This field value is set to zero at the factory and cannot be reset. The Note field is a six-character, alphanumeric, user-programmable field.

❖ To enter the last service date or other comments

In the Note field, press the [+] or [–] keys.

Figure 105. Total System Cycles menu



Injector Valve Menu

Except for the Total System Cycles menu, all of the Maintenance Log menus are identical to the Injector Valve menu (see Figure 106 and Table 22). Only the Injector Valve menu then is described in detail here.

The Maintenance log menus track the usage for the listed autosampler component. When you replace the component, reset the counter to 0.

❖ To reset the counter to 0

1. Press the [+] or [–] keys to select **YES**.
2. Press ENTER to accept the new setting.

   The **Counter Reset** confirmation message appears.

Figure 106. Injector Valve menu

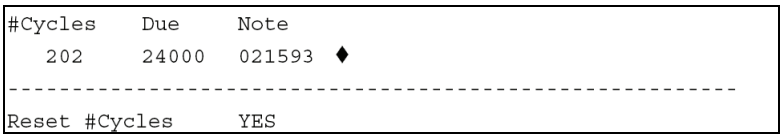


Table 22. Maintenance Log menu parameters

#Cycles	Displays the number of activations of the specific autosampler part since the last time this field (counter) was reset (0 to 999 999). The #Cycles value for each of the Maintenance Log menus is included in the menu descriptions that follow.
Due	Specifies a six-character numeric field where you enter the number of cycles before maintenance is due. Range: 0 to 999 999 If the #Cycles field value is larger than the Due field value, the Maintenance Due message appears when you access the Maintenance Log menu from the Options menu. The default maintenance Due field value is 12 000 (approximately once a year). The default values for the other Maintenance Log menus are included in the menu descriptions that follow.
	Tip The Due field default values for all of the Maintenance Log menus are estimates only. The actual values appropriate for your instrument vary with the type of applications and the specific conditions being used with your LC. If the "Maintenance Required" message is displayed, strongly consider replacing parts; however, you might want to increase the Due value and experiment with extending the time before performing maintenance. Conversely, if instrument performance indicates, you might want to decrease the Due field value for some of the Maintenance Log menus.
	Tip Similar to the Total System Cycles menu, this is a six-character, alphanumeric, user-programmable field. Use the [+] or [–] keys to enter the last service date in this field, but you might also document contact information or other comments regarding the maintenance of the specific autosampler part.
Reset #Cycles	Pressing the DOWN ARROW key (▼) displays the Reset #Cycles field used to reset the #Cycles counter for each autosampler part.

Needle Wear Menu

The #Cycles field in this menu is incremented each time the needle pierces a vial. The default value of the Due field is 10 000, which corresponds to approximately every six months.

Sample Syringe Menu

The #Cycles field displays each full syringe stroke. However, the instrument tracks the actual distance of linear travel, thus compensating for different injection (stroke) volumes. The default Due field value for both menus is 24 000, which corresponds to approximately once a year.

Troubleshooting

This chapter provides information to help you troubleshoot possible autosampler problems.

Contents

For additional assistance in troubleshooting a particular problem, contact Thermo Fisher Scientific Technical Support or your service representative.

General Chromatography Troubleshooting

The first section focuses on general chromatographic problems and is designed to help you define the particular instrument or condition causing the problem. Once you determine the cause, use the ordered outline to fix the problem or refer to the troubleshooting guide for the specific SpectraSYSTEM instrument. Each of these problems can be detected by monitoring the chromatography running conditions or by observing the chromatogram.

Table 23. General LC troubleshooting (Sheet 1 of 3)

Symptom	Cause/Remedy
1. No flow	a. Check mobile phase connections.
	b. Check for leaks.
	c. Refer to the pump troubleshooting guide.
2. High backpressure	a. Check flow rate and system/column specifications.
	b. Check for tubing or column blockage.
	c. Refer to the pump troubleshooting guide.
3. Unstable baseline or drift	a. System/column not equilibrated; allow more time.
	b. Refer to the detector troubleshooting guide.
	c. Refer to the pump troubleshooting guide.

Table 23. General LC troubleshooting (Sheet 2 of 3)

Symptom	Cause/Remedy
4. Baseline noise	a. Check for air bubbles in system, degas solvents.
	b. Check for system/solvent contamination.
	c. Refer to the pump troubleshooting guide.
	d. Refer to the chromatography data system troubleshooting guide.
5. No peaks	a. Check detector and data system connections.
	b. Refer to the autosampler troubleshooting guide.
	c. Check sample retention with chromatographic conditions.
6. Contaminating/ghost peaks	a. Clean system and column.
	b. Refer to the autosampler troubleshooting guide.
	c. Refer to the pump troubleshooting guide.
7. Poor peak shape	a. Check system for leaks.
	b. Check fittings and tubing lengths.
	c. Check column performance.
	d. Refer to the autosampler troubleshooting guide.
	e. Refer to the pump troubleshooting guide.
	f. Refer to the detector troubleshooting guide.
8. Poor retention time reproducibility	a. Check system for leaks and bubbles.
	b. System/column not equilibrated; allow more time.
	c. Check column performance.
	d. Refer to the pump troubleshooting guide.
	e. Refer to the autosampler troubleshooting guide.
	f. Refer to the data system troubleshooting guide.
9. Poor peak area reproducibility	a. Check column performance.
	b. Refer to the autosampler troubleshooting guide.
	c. Refer to the data system troubleshooting guide.
10. Non-integrated or too many peaks	a. Refer to the integrator or data system troubleshooting guide.

Table 23. General LC troubleshooting (Sheet 3 of 3)

Symptom	Cause/Remedy
11. No instrument of device control	a. Check cable connections.
	b. Check system configuration.
	c. Refer to the individual instrument troubleshooting guide.
	d. Refer to the integrator, ISM, or data system troubleshooting guide.

For more detailed chromatographic troubleshooting, refer to any HPLC troubleshooting reference book or call your local sales or service representative.

Autosampler Troubleshooting

This section describes autosampler problems that can affect your chromatography and general autosampler hardware problems.

For information on troubleshooting autosampler problems that can affect your chromatography, see Table 24. For information on troubleshooting general autosampler hardware problems, see Table 25.

Table 24. Autosampler-specific chromatographic problems (Sheet 1 of 3)

Symptom	Possible problem	Remedy
1. Baseline drift when the injection valve changes from INJECT to FILL	a. Lack of thermal stability in the column oven compartment, detector, or mobile phase	a. Eliminate drafts around the column compartment. Wait until the column oven has reached thermal equilibrium. If the problem persists, contact your local Thermo Fisher Scientific service representative.
2. Shifting retention times	a. Temperature variations in the lab	a. Stabilize temperature.
	b. Column-heater problems	b. Call your Thermo Fisher Scientific service representative.
3. Sensitivity changes	a. Inconsistent sample injection volumes	a. Check for a partially blocked injection valve and observe the pressure. If the problem persists, replace the injection valve (see "Removing the Injection Valve" on page 110).
	b. Leaks between the injection valve and the column causing inconsistent sample-volume injection	b. Tighten/remake fitting.
	c. Air bubbles aspirated into the lines along with sample	c. Replenish/prepare fresh sample and repeat the injection.
		c. Slow down syringe speed.
	e. Sample adsorption to the walls of the injection valve or sample loop	e. Change the mobile phase concentration.
	f. Sample solvent incompatible with the mobile phase	f. Change solvent; use mobile phase if possible.
	g. Sample insoluble in solvent	g. Be sure sample solvent has a lower elution strength than the mobile phase.
	h. Air in syringe/sample lines	h. Flush sample lines.

Table 24. Autosampler-specific chromatographic problems (Sheet 2 of 3)

Symptom	Possible problem	Remedy
4. Irregular peak shapes	a. The sample volume exceeds the column's capacity.	a. Decrease the volume injected or dilute the sample.
	b. The strength of the solvent used to dilute the sample exceeds the strength of the initial mobile phase.	b. If appropriate for your application, ensure that the flush solvent is of the same composition as the solvent used to dissolve the sample and that it never exceeds the solvent strength of the initial mobile phase.
	c. Degradation of sample components	c. Prepare fresh sample and repeat injection.
5. Band broadening	a. Fitting problem at the injection valve (port 5) to column connection	a. Inspect for damage, mismatch, incorrect assembly.
	b. Tubing ID too large	b. Use 0.010 inch ID tubing.
6. Smaller than expected peak heights	a. Smaller than expected injection volume due to incorrect syringe configuration	a. Check the syringe size and the syringe configuration.
	b. Incorrect injection volume	b. Check the injection volume value in the file.
	c. Air in lines	c. Flush the lines.
	d. Misadjusted syringe-drive mechanism	d. Call your Thermo Fisher Scientific service representative.
7. Peaks during a blank injection (from a previous injection)	a. Sample carryover due to residual sample in the sample loop	a. Insufficient flush volume. Increase Injection Volume in Edit menu, add a blank vial between sample vials, or use a stronger flush solvent.
	b. Sample carryover due to incorrectly assembled sample-loop fittings (adding dead volume that can contain residual sample)	b. Check and remake fittings.
	c. Sample too concentrated	c. Dilute sample.
8. No peaks	a. Plugged or bent needle	a. Clean/replace needle. Tighten fittings and syringe.
	b. Leaking flush valve fitting	b. Tighten fitting.
	c. Plugged or leaking tubing	c. Replace faulty tubing.

Table 24. Autosampler-specific chromatographic problems (Sheet 3 of 3)

Symptom	Possible problem	Remedy
9. Incorrect precision and/or accuracy problem on a sample of known content-random error	a. Air leak	a. Tighten all fittings and repeat the run.
	b. Worn out syringe	b. Replace.
10. Reproducibility of peak areas	a. Plugged or bent needle	a. Clean/replace needle. Tighten fittings and syringe.
	b. Leaking flush valve fitting	b. Tighten fitting.
	c. Plugged or leaking tubing	c. Replace faulty tubing.
	d. Sample viscosity too low or too high	d. Change viscosity setting in the FILE > Edit > More menu.
	e. Solvent viscosity too low or too high	e. Change Solv Viscosity setting in the OPTIONS > Configurations menu.

Table 25. Autosampler hardware problems (Sheet 1 of 4)

Symptom	Possible problem	Remedy
Startup		
1. Nothing works when instrument is turned on.	a. Power cord unplugged or faulty	a. Plug in/replace cord.
	b. Blown fuse	b. Replace fuse.
	c. Defective power switch, driver, transformer	c. Call your local Thermo Fisher Scientific service representative.
2. Fan is running but display does not work.	a. Display component failure	a. Call your local Thermo Fisher Scientific service representative.
3. No response from keypad but display works.	a. Defective keypad	a. Call your Thermo Fisher Scientific service representative.
4. Column oven, tray temperature control, or both do not turn on.	a. File not loaded	a. Load file.
Communications		
5. No RS-232-C communications.	a. Further troubleshooting required	a. Call your Thermo Fisher Scientific service representative.
6. EEPROM fails to retain memory.	a. EEPROM not initialized	a. Reinitialize the EEPROM. If unsuccessful, might be a defective PCB. Call your Thermo Fisher Scientific service representative.
7. External devices (timed events) do not work.	a. Incorrect configuration settings or hardware connections	a. Check "Communication: SpectraNet or Stand Alone" on page 30 for configuration settings and hardware connections.
	b. Interface equipment problem	b. Refer to the applicable instrument manuals.
Vials		
8. Vial is stuck in tower.	a. Bent needle	a. Replace needle module (see "Maintaining the Sample Needle Module" on page 115.)

Table 25. Autosampler hardware problems (Sheet 2 of 4)

Symptom	Possible problem	Remedy
9. Instrument cannot locate vial.	a. Incorrect vial position entered into Samples menu	a. Check Samples menu.
	b. Sample incorrectly placed in sample tray	b. Check tray arrangement.
	c. Arm misaligned	c. Home arm. See "Special Commands in the Commands Menu" on page 96.
	d. Arm movement restricted	d. Remove restriction and home arm.
10. Vials do not fit in tray.	a. Incorrect vials	a. Vial diameter should be 0.44 to 47 inches.
11. Broken vials	a. Tray not aligned or not seated properly	a. Seat tray completely in tray chamber and pull handle back toward you.
12. Torn septa	a. Bent needle	a. Replace needle.
Injection valve		
13. Injection valve does not switch.	a. Slipping injection valve drive	a. Call your Thermo Fisher Scientific service representative.
14. Injection valve continues to rotate until fault is generated.	a. Loose or faulty sensor cable	a. Call your Thermo Fisher Scientific service representative.
15. Injection valve does not rotate to the proper position.	a. Excessive torque required for valve rotation	a. Call your Thermo Fisher Scientific service representative.
16. Motor fails to stop after syringe is fully returned.	a. Possible defective sensor	a. Call your Thermo Fisher Scientific service representative.
17. Sample loop does not fill.	a. Blocked injection valve	a. To isolate blockage and flush, follow procedures on "Replacing the Flush Solvent Inlet Filter" on page 115. Or, take apart valve and sonicate.
		CAUTION Do not clean valve with a cleaning wire, or you risk damaging it.
	b. Blocked sample loop	b. Backflush loop. If problem persists, replace the loop.
	c. Worn syringe	c. Substitute a "known-good" syringe and determine if symptoms persist.
	d. Blocked needle	d. Clean with a cleaning wire.

Table 25. Autosampler hardware problems (Sheet 3 of 4)

Symptom	Possible problem	Remedy
18. Valve-seal wears rapidly.	a. Too much valve seal pressure against valve	a. Decrease LC system pressure.
	b. Particulate matter in valve	b. Use an inline filter before the injection valve.
	c. Inadequately filtered sample	c. Filter with a 0.5 µm filter before injection.
	d. Buffer crystallization	d. Do not allow buffers to stand in system. Run a flush sequence from the Commands menu when the system is not in use.
19. Injection from incorrect vial.	a. Incorrect vial position entered in Samples menu	a. Check Samples menu.
	b. Sample placed in incorrect position in vial tray	b. Check tray arrangement.
20. Samples are injected incorrectly in a pattern.	a. Error in file (calibration vials placement)	a. Check file (calibration template).
Needle		
21. Needle is bent or broken.	a. Incorrect vials	a. See list of recommended replacement parts in the front of the manual.
	b. Alignment problem	b. Call Thermo Fisher Scientific.
	c. Arm sticking	c. Call your Thermo Fisher Scientific service representative.
22. Needle is plugging.	a. Multiple/incorrect septa	a. Verify a single septum of the type recommended.
Syringe		
23. Syringe drive creates loud buzzing.	a. Defective limit switch	a. Call your Thermo Fisher Scientific service representative.
24. Flush valve will not activate.	a. Defective valve/coupler/motor	a. Call your Thermo Fisher Scientific service representative.
25. Flush valve is actuated, but syringe cannot draw flush solvent.	a. Plugged inlet filter in solvent reservoir	a. Replace inlet filter (see "Replacing the Flush Solvent Inlet Filter" on page 115).
Arm		
26. Hook does not lift vial.	a. XYZ arm motor stepping failure	a. Call your Thermo Fisher Scientific service representative.

Table 25. Autosampler hardware problems (Sheet 4 of 4)

Symptom	Possible problem	Remedy
Leaks		
27. Leakage during loop filling	a. Blocked loop or valve not deflected far enough	a. Flush sample loop or replace; check and flush waste line. Check the sensor.
28. Cross-port leakage	a. Worn rotor seal	a. Replace according to instructions in "Replacing the Rotor Seal" on page 109.
29. Liquid on vial caps	a. Leaky flush valve	a. Replace flush valve.
	b. Leaky injection valve	b. Replace rotor seal (see "Installing the Rotor Seal" on page 112).
Other		
30. An automatic sequence is interrupted, or instrument will not start.	a. Column-oven, heater, door interlock activated	a. Check the Ready Participation menu.
31. In Stand Alone mode, Status Screen indicates a run in progress, but the instrument is locked up.	a. Configuration incorrectly set to SpectraNet mode	a. In the Configurations menu, set Mode to Stand Alone.
32. Sample is contaminated.	a. Dirty vials	a. Replace vials.
	b. Improper septa made of silicone or other rubber-like material	b. Use recommended septa. Thermo Fisher Scientific septa are made of Teflon-faced silicone.
33. System pressures are elevated.	a. Block between column and autosampler	a. Crack each fitting and observe pressure. See instructions in "Testing for Tubing Restrictions" on page 144.

Display Messages

As mentioned in Chapter 2, "Installation," three different kinds of messages can appear on your autosampler display: user messages, confirmation messages, and error messages. This section describes the possible conditions that generate these messages.

- User Messages
- Confirmation Messages
- Error Messages

User Messages

User messages are displayed as a result of the user's incorrect action.

User message	Description
Autosampler Busy	You cannot issue a command from a menu or press a key while the autosampler is in the middle of a previously requested operation.
Autosampler Busy No Loading Allowed	You cannot load a sample set while the queue is running.
Cannot Add Priority Set During Final Calibration	You cannot add a priority set during bracketing (after all the sample vials have been injected).
Cannot Add Priority Set at This Time	You cannot add a priority set to other than set 1. You cannot add a priority set unless the file's Calibration menu calls for interspersed calibration vials (you must select Reuse calibration vials if you want to add priority sets to the queue).
File in Queue Cannot Be Copied To	You cannot copy to a file assigned to a sample set in the queue.
File In Queue Cannot Be Deleted	You cannot delete a file assigned to a sample set in the queue.
File In Queue Cannot Be Edited	You cannot edit a file assigned to a sample set in the queue from the Edit menu. You can edit the run-file copy of the active file from the Samples menu.
File Protected Cannot Be Copied To	You cannot copy to a file that is protected (Protect is set to On) in the Configurations menu.
Use Samples Key to Add Samples to Queue	You cannot start a run by pressing the RUN key when the queue is empty. You must first load or add sample sets to the queue.

Confirmation Messages

Confirmation messages inform you that the autosampler is completing a requested operation or command. These messages disappear when the operation is complete.

Confirmation message	Description
Aligning Arm	The Align Arm command is in progress.
Clearing Mixer	The Clear Mixer command is in progress.
File Copied	The requested file has been copied.
File Deleted	The requested file has been deleted.
File Loaded	The requested file has been loaded into the queue.
Flush Sample Syringe	The Flush Sample Syringe command is in progress.
Homing Arm	The Home Arm command is in progress.
Initializing	The Initialize Hardware command is in progress (see"Initialize Hardware" on page 97).
Set Added to Queue	The requested sample set has been added to the sample queue.

Error Messages

There are three kinds of errors: hardware errors, system errors, and diagnostic errors.

This topic describes these types of errors:

- "Hardware Errors" on page 140
- "System Errors" on page 143

For descriptions of diagnostic errors, see "Diagnostics in the Tests Menu" on page 101.

Hardware Errors

Hardware errors occur during the normal use and operation of the instrument. Most of the time you can clear these errors. For some, you might need to call your Thermo Fisher Scientific service representative.

To clear the error message, press any key, and then take the steps suggested for each message in Table 26 to resolve the problem.

Table 26. Hardware error messages (Sheet 1 of 3)

Hardware error message	Description
Arm Failed to Home on X, Y, Z	The XYZ arm could not make the final *x*-, *y*-, or *z*-axis motion required to return to the home position. Check for a dislodged vial, tray, or other obstruction. Run the Initialize Hardware routine. If the error persists, contact your Thermo Fisher Scientific service representative.
Arm Jam on X, (Y, Z) Axis	The XYZ arm is obstructed on its left-to-right (*x*) axis, its front-to-back (*y*) axis, or its vertical (*z*) axis. Check for a dislodged vial, tray, or other obstruction. Run the Initialize Hardware routine. If the error persists, contact your Thermo Fisher Scientific service representative.
Flush Valve Misaligned	The flush valve did not turn all the way to the required position. Perform the Initialize Hardware routine. If the error persists, run the Test Connectors routine. If the error persists, contact your Thermo Fisher Scientific service representative.
Hardware Failure	A problem occurred with the valves or syringes. Run the Initialize Hardware diagnostic (see "Initialize Hardware" on page 97), the Injector Test (see "Injector" on page 97), or both. If you are unable to clear the error, contact your Thermo Fisher Scientific service representative.
Hardware Not Homed	The solvent valve could not move to a certain requested position, but the hardware was not initialized properly. Or, on power-up, the solvent valve could not home properly. Run the Initialize Hardware diagnostic (see "Initialize Hardware" on page 97). If you are unable to clear the error, contact your Thermo Fisher Scientific service representative.
Hardware Timeout	Time ran out for completing the requested mechanical operation. The problem could be with the arm, valves, or syringes. Run the Initialize Hardware routine (see "Initialize Hardware" on page 97). If the problem persists, try manually flushing the syringes using the Flush Sample and Flush Prep Syringe commands (see "Flush Sample Syringe" on page 96). If the problem persists, contact your Thermo Fisher Scientific service representative.

Table 26. Hardware error messages (Sheet 2 of 3)

Hardware error message	Description
Injector Valve Failure	The injection valve failed to move (between the INJECT and FILL positions, or vice versa) for the requested operation. Issue the Injector command from the Commands menu. If the error persists, call your Thermo Fisher Scientific service representative.
Inject Valve Misaligned	The injection valve is positioned between the INJECT and FILL positions. Run the Injector test (see "Injector" on page 97), and call your Thermo Fisher Scientific service representative if the error persists.
IValve Move Is Impossible	The injection valve cannot move to the requested position from its current position. Switch the injection valve position using the Injector command, and call your Thermo Fisher Scientific service representative if the error persists.
IValve Move Not Detected	The system did not detect a requested movement to the required position. Check for a loose connection to the injection valve. Try manually moving the injection valve to another position. If the error persists, call your Thermo Fisher Scientific service representative.
Inject Valve Overshoot	The injection valve moved past the desired position. Run the Injector test (see "Injector" on page 97), and call your Thermo Fisher Scientific service representative if the error persists.
Injector Valve Undershoot	The injection valve failed to move far enough to the desired position. Run the Injector test (see "Injector" on page 97), and call your Thermo Fisher Scientific service representative if the error persists.
Parameter Out of Range	You entered a value that was too large or too small. For example, you entered an injection volume that was too large for the installed syringe. Check the installed syringe/sample loop versus the edit file parameters.
Syr 1(2) Not Enough to Expel	The syringe is not retracted far enough to expel the requested volume flush operation. Run the Initialize Hardware test (see "Initialize Hardware" on page 97), and restart the flush cycle.

Table 26. Hardware error messages (Sheet 3 of 3)

Hardware error message	Description
Syringe Failure	The syringe failed to move to the appropriate position required for an operation. Run the Initialize Hardware test (see "Initialize Hardware" on page 97). If the error repeats, call your Thermo Fisher Scientific service representative.
Syringe Overfill	You requested an injection volume that is too large for the size of the installed syringe. Check the method and syringe volume.
Unexpected Vial in Hook	A vial was found in the hook where no vial was expected for the requested operation. To clear the error, remove the vial and restart or resume the requested operation.
Vial Not Found in Hook	No vial was found in the hook where one was expected. Verify that a vial exists in the requested tray location, check the file and vial assignments in the Samples menu, and check to see if a vial is lodged in the sample tower. (If so, the needle might be bent.)

System Errors

System errors occur very rarely but they cancel normal operation. Because system errors are not usually problems you can correct, they are listed in this section but not described. If you see these or other messages, document them and contact your Thermo Fisher Scientific service representative for assistance.

Invalid Current Location

Invalid Command

Not in GET/RET Sequence

Z Must be Up/No Vial

Move Z Not Allowed

Get LocNum: Bad Row, Col

Z up Only in Serv Block

Invalid Target Location

SValve Extra Interrupt

Bad Vial Number

Unexpected Syr Event

Testing for Tubing Restrictions

If the solvent lines are restricted, you will probably hear a loud grinding sound from the syringe-drive mechanism due to increased resistance to syringe travel.

IMPORTANT Before beginning this procedure, be sure to set up a beaker or other container to catch the solvent.

❖ To isolate a block to a section of tubing

1. Place the autosampler injection valve into the FILL position (press the MENU key and select **COMMANDS > Injector > Rotate Injection Valve**, and press the [+] and [–] keys to select **Fill**).
2. Disconnect the tubing from port 3 of the injection valve.
3. Initiate a flush sequence (press the MENU key and select **COMMANDS > Flush Sample Syringe** (see "Flush Sample Syringe" on page 96). Solvent flows freely on the return stroke of the plunger if there are no restrictions present. Flow restriction is unlikely at this point due to the large internal tubing diameter.
4. Reconnect the tubing.
5. Disconnect the needle and check the flow.
6. If no restriction is found, reattach all of the tubing, and flush the syringe to verify that the restriction still exists.
7. If a restriction is still present, remove and clean the sample needle as described in "Maintaining the Sample Needle Module" on page 115.

Replaceable Parts

When you must replace parts for use with your SpectraSYSTEM autosampler, refer to this list of spare parts, consumables, and kits available from Thermo Fisher Scientific.

Contents

Clear Vials and Vial Kits

Shell Vials

Shell vials, 100/package A4946-010
Shell Vial Kit A4948-010

Standard Vials

Standard vials, 100/package A4951-010
Standard vial septa, 100/package. A4952-010
Standard vial caps, 100/package A4953-010
Standard Vial Kit, 100/package A4954-010

Crimp Vials/Caps

Crimp caps, 100/package A4955-010
Crimp vials, 100/package, 1.8 mL A4956-010
Crimp Vial Kit, 100/package A4957-010

Poly Kits

Polypropylene Kits, 600 mL, 100/package A4958-010
Polypropylene Kits, 100 mL, 100/package A4959-010

Inserts

Spring for insert, 100/package A4981-010
Insert, 50 mL, 100/package A4960-010
Insert, 100 mL, 100/package A4961-010

Amber Vials and Vial Kits

Crimp Vials

Amber crimp vials, 100/package A4964-010
Amber Vial Crimp Kit, 100/package A4965-010

Standard Vials

Standard amber vials, 100/package A4966-010
Standard Amber Vial Kit, 100/package A4967-010

PFTE Liners/Septa

PTFE Liners, 100/package A4968-010
PTFE Vial caps/septa, 100/package A4969-010
Standard vial station A4970-010

Sample Trays

Three sample trays, 35-vial capacity A3696-010

Cables and Adapters

Autosampler-to-integrator cable/adapter A3981-010
BCD Communications option
(This option requires field installation by Thermo Fisher Scientific unless purchased simultaneously with a SpectraSYSTEM autosampler.) A3663-010

Standard Autosamplers

Injector valve assembly for the AS3000, 100 mL, stainless steel
(Assembly includes the complete autosampler valve assembly.) A3566-030
Standard Maintenance Kit (Kit includes everything necessary to maintain the instrument for one year: inlet filter, needle replacement kit, valve rotor seal, syringe, lubricant, cleaner, and LC test sample.) A4101-010
Standard Fittings Kit (Kit includes stainless steel fittings and tubing used in a SpectraSYSTEM LC system.) A4051-010
Autosampler Needle Replacement Kit
(Kit includes the standard, stainless-steel, deflected-point replacement needle.) A4719-010

Standard Sample Loops and Syringes

Rheodyne sample loop, 20 µL, stainless steel 3302-0230
Rheodyne sample loop, 50 µL, stainless steel 3302-0010
Rheodyne sample loop, 100 µL, stainless steel 3302-0020
Rheodyne sample loop, 200 µL, stainless steel 3302-0220
Rheodyne sample loop, 500 µL, stainless steel 3302-0030
Rheodyne sample loop, 1 mL, stainless steel 3302-0040
Screw-tip syringe, 250 µL, (standard) A3588-020
Screw-tip syringe, 500 µL A3588-010
Solvent inlet filter cartridge (pkg. of four) A4094-010

Inert/Biocompatible Autosamplers

Injector Valve Assembly
(The assembly includes the complete autosampler valve assembly.) A3566-020
Inert/Biocompatible Maintenance Kit
(The kit includes the inert/biocompatible versions of everything in the Standard Maintenance Kit.) A4102-010
Inert/Biocompatible Fittings Kit
(The kit includes PEEK fittings and tubing used in an inert/biocompatible SpectraSYSTEM LC system.) A4061-010
Autosampler Needle Replacement Kit (Kit includes the standard, inert, deflected-point replacement needle.) A4719-020

Inert/Biocompatible Sample Loops and Syringes

Rheodyne Sample Loop, 20 µL. A4169-010
Rheodyne Sample Loop, 50 µL. A4169-020
Rheodyne Sample Loop, 100 µL. A4169-030
Rheodyne Sample Loop, 200 µL. A4169-040
Rheodyne Sample Loop, 500 µL. A4169-050
Rheodyne Sample Loop, 1 mL A4169-060
Rheodyne Sample Loop, 2 mL A4169-070
Screw-tip Syringe, 250 µL. A3588-020
Screw-tip Syringe, 500 µL. A3588-010

Solvent Inlet Filter Cartridge (pkg. of four) A4094-010

Test Mixes

Autosampler Test Mix A4991-010
Autosampler Dilution Test Mix. A5135-010

Upgrade Kits

Upgrade kits are available for SpectraSYSTEM stainless steel autosamplers. Contact your local Thermo Fisher Scientific representative for details.

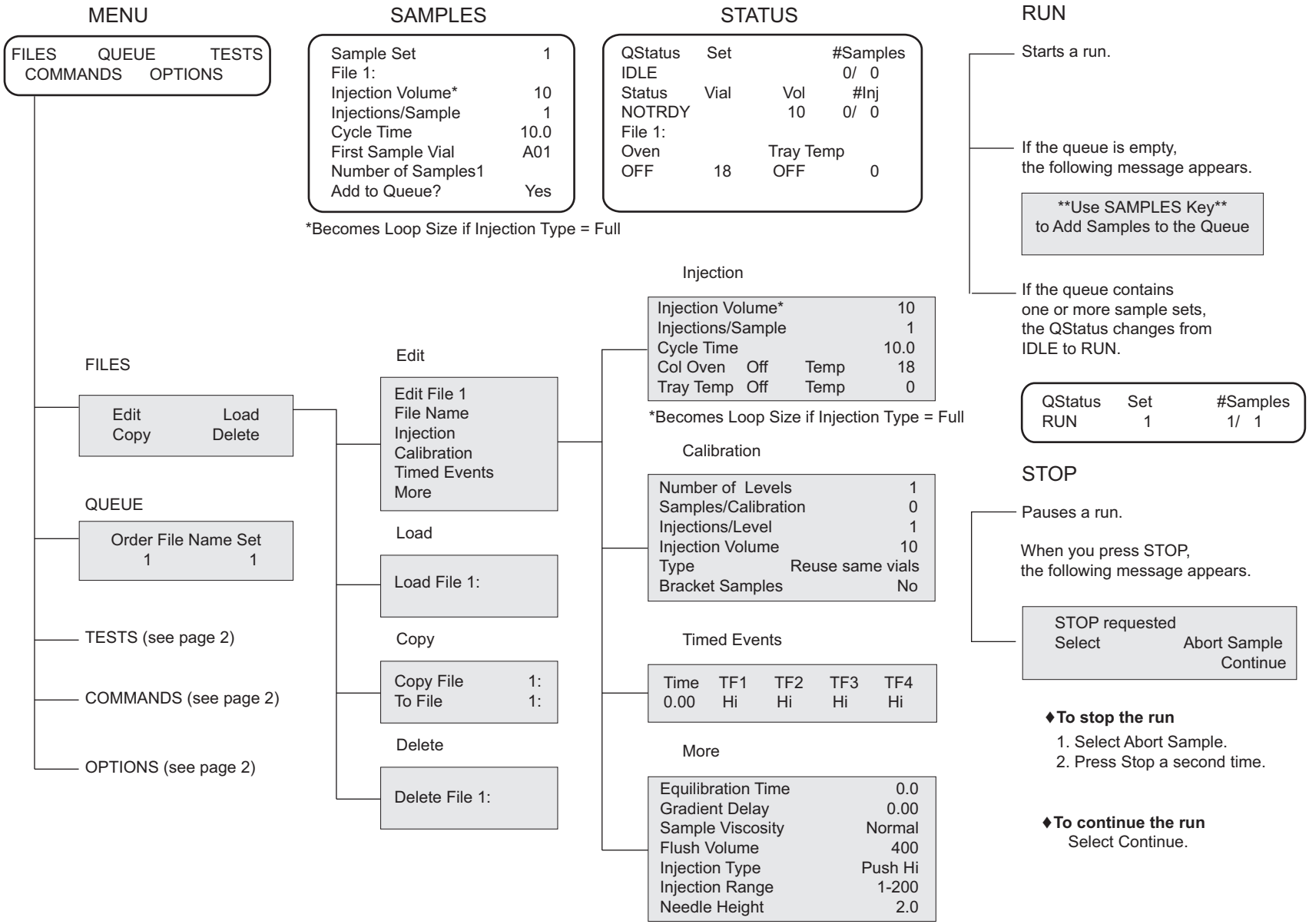
Quick Reference Guides

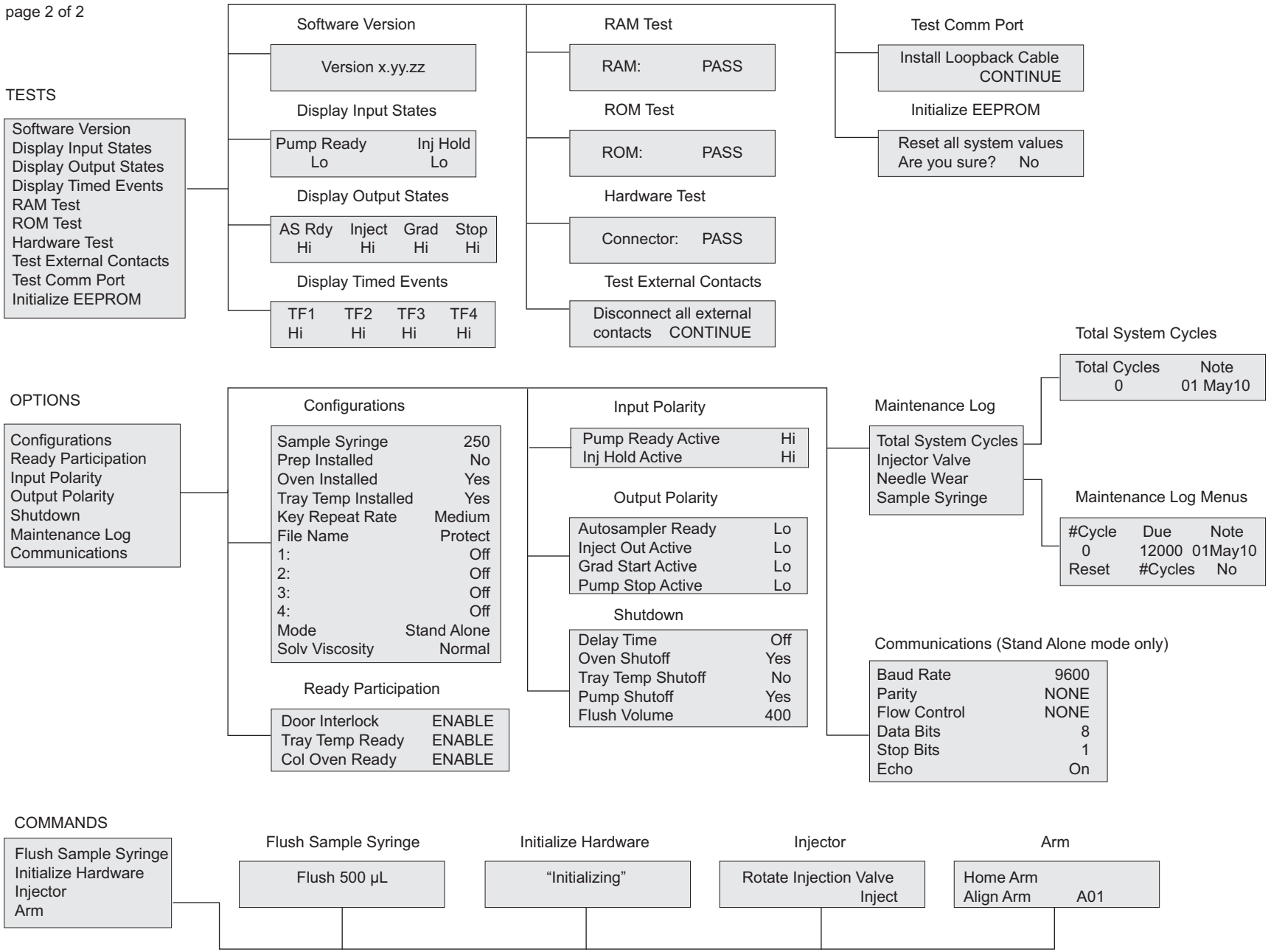
Use the following two-page guides to familiarize yourself with the SpectraSYSTEM AS3000 autosampler's command center.

Tip To control the autosampler from the command center, set the **Options > Configurations > Mode** field to Stand Alone.

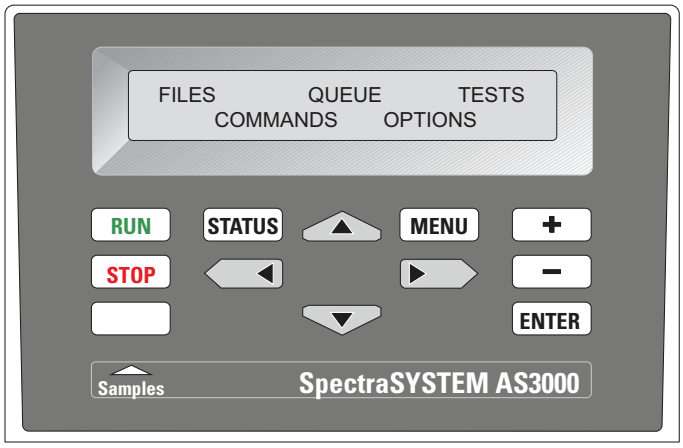
Contents

SpectraSYSTEM AS3000 Menus




TESTS
Software Version
Display Input States
Display Output States
Display Timed Events
RAM Test
ROM Test
Hardware Test
Test External Contacts
Test Comm Port
Initialize EEPROM
Software Version
Version x.yy.zz
Display Input States
Pump Ready Inj Hold
Lo Lo
Display Output States
AS Rdy Inject Grad Stop
Hi Hi Hi Hi
Display Timed Events
TF1 TF2 TF3 TF4
Hi Hi Hi Hi
RAM Test
RAM: PASS
ROM Test
ROM: PASS
Hardware Test
Connector: PASS
Test External Contacts
Disconnect all external contacts CONTINUE
Test Comm Port
Install Loopback Cable CONTINUE
Initialize EEPROM
Reset all system values
Are you sure? No
OPTIONS
Configurations
Ready Participation
Input Polarity
Output Polarity
Shutdown
Maintenance Log
Communications
Configurations
Sample Syringe 250
Prep Installed No
Oven Installed Yes
Tray Temp Installed Yes
Key Repeat Rate Medium
File Name Protect
1: Off
2: Off
3: Off
4: Off
Mode Stand Alone
Solv Viscosity Normal
Ready Participation
Door Interlock ENABLE
Tray Temp Ready ENABLE
Col Oven Ready ENABLE
Input Polarity
Pump Ready Active Hi
Inj Hold Active Hi
Output Polarity
Autosampler Ready Lo
Inject Out Active Lo
Grad Start Active Lo
Pump Stop Active Lo
Shutdown
Delay Time Off
Oven Shutoff Yes
Tray Temp Shutoff No
Pump Shutoff Yes
Flush Volume 400
Maintenance Log
Total System Cycles
Injector Valve
Needle Wear
Sample Syringe
Total System Cycles
Total Cycles Note
0 01 May10
Maintenance Log Menus
#Cycle Due Note
0 12000 01May10
Reset #Cycles No
Communications (Stand Alone mode only)
Baud Rate 9600
Parity NONE
Flow Control NONE
Data Bits 8
Stop Bits 1
Echo On
COMMANDS
Flush Sample Syringe
Initialize Hardware
Injector
Arm
Flush Sample Syringe
Flush 500 µL
Initialize Hardware
“Initializing”
Injector
Rotate Injection Valve Inject
Arm
Home Arm
Align Arm A01

SpectraSYSTEM AS3000 Quick Reference Guide



Using the Keypad

RUN	Starts a run.
STOP	Pauses the sample queue. You can continue the run from the current vial or cancel the run and return to the beginning of the sample queue.
Samples	Opens a menu where you can specify the injection parameters and a file name for a sample set, add sample sets to the queue, and edit runs in progress.
STATUS	Displays the status screen.
▲, ▼, ◀, and ▶	Moves the cursor in the direction indicated on the key. The UP and DOWN arrow keys also move the cursor between menus and displays.
MENU	Displays the main menu. For information on the menus, refer to the AS3000 Menus sheet.
+ and −	Scrolls you through a field's available choices or changes the value of alphanumeric entries.
ENTER	Accepts a selection or menu entry; also advances the cursor to a new field.

Setting Parameters

❖ To enter values

- For numeric entries, use the + or − key to increase or decrease the value of each digit.
- For alphanumeric entries, use the + and − keys to scroll through the alphabet from A to Z, then through numbers 0 to 9, and then slash, hyphen, and blank space.
- For preset choices, use the + key to advance through a preset list of choices. The − key takes you back through the list.

❖ To accept values

Press the ENTER, MENU, or STATUS keys or use the arrow keys.

Installing Trays

Trays A, B, and C are arranged from left to right in the sample tub. The position number is indicated on the top left of each vial position.

❖ To install your trays

1. Hold the tray handle and tilt the back end down.
2. Insert the tray's runner into the slots at the back of the sample tub and lower the front of the tray into place.
3. Press down firmly to seat the tray and pull the handle back toward you to be sure the tray is properly seated.

Managing Files

❖ To set up a file

1. Press MENU and select **FILES > Edit**.
2. Enter the file number and name.
3. Select **Injection**.
4. Enter the injection volume, number of injections per vial, and cycle time, and set the oven and sample tray temperatures.
5. Select **Calibration**.
6. Enter the number of standard levels, calibration interval, number of injections per level, and injection volume. Select the type of calibration routine (reuse or intersperse calibration vials). Select whether or not (yes or no) you want the sample set to end with a calibration set.
7. To program external events, select **Timed Events** and program a time and state (**Hi** or **Lo**) for up to four time functions.
8. Select **More**.
9. Select the injection type: **Full**, **Pull**, **Push Hi**, or **Push Lo**. Enter the equilibration time, gradient delay, flush volume, and needle height values for your application.

❖ To load a file

1. Press MENU and select **FILES > Load**.
2. Select the file number and press ENTER.

❖ To build the sample queue

1. Press the Samples key.
2. Enter the file number in the Use File field.
3. Press ENTER to accept the other values in the Samples Menu and to add the file to the Queue (Yes).

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❖ To run a file

1. Press RUN to start the run.
2. To monitor the sample queue, vial injection, and column and tray temperatures, press STATUS.
3. To pause a run, press STOP.

   STOP requested appears.
4. Do one of the following:
   - To cancel the run, select **ABORT Sample**, and then press STOP again.
   - To continue the run, select **Continue**.

❖ To edit a run in progress

You can edit some of the file parameters for the run in progress from the Samples menu.

1. Press the Samples key. Press ▼.
2. Edit the Injection Volume, Injections/Sample, Cycle Time, and Number of Samples values.

   You can edit the Calib 1 (of *X*) value only if you have selected Reuse vials as your calibration routine. You cannot edit the First Sample Vial value.

   Except for the Cycle Time and Calib 1 (of *X*) values, changes take place on the next sample injection. A cycle time change affects the current sample injection, a Calib 1 (of *X*) change affects the next recalibration.

❖ To delete a file

1. Press MENU and select **FILES > Delete**.
2. Select the file number to be deleted.
3. Press ENTER.

❖ To copy a file

1. Press MENU and select **FILES > Copy**.
2. Select the file number to be copied from the Copy file field, and the file number to be copied to the To file field.
3. Press ENTER.

❖ To protect a file

1. Press MENU and select **OPTIONS > More**.
2. Scroll down to the File Name and Protect fields. Select **On** in the appropriate file's Protect field.
3. Press ENTER.

Managing the Queue

❖ To reorder sets in the Queue

1. Press MENU and select **QUEUE**.
2. Move the cursor to the line where you want the change to occur and increment or decrement to the new Order value.
3. Move the cursor out of the field to automatically rearrange the lines.

❖ To add a set to the queue

1. Press the Samples key.
2. Enter the file number and set to add to the queue.
3. Accept the Edit file parameters and press ENTER in the Add to Queue? field.

❖ To delete a set from the queue

1. Press MENU and select **QUEUE**.
2. Move the cursor to the line to be deleted.
3. Decrement the Order value to **0** and then blank. Moving the cursor deletes the blank line from the display.

❖ To add a priority set

A priority sample set is inserted into the active (currently running) run before other samples in the set. It uses the same file name as the active set.

Note When running priority sets, remember the following:

- If you are running calibration vials in the priority set, you must select Reuse vials as your calibration routine.
- You cannot change the order of a priority set in the queue.

1. Press the Samples key.
2. Use the − key to change the Set field value to **P**.
3. Fill in the other file parameters as for any other set.
4. Add the priority set to the queue.
5. Press MENU and select **QUEUE**. The priority set has an Order value of 1, has the same File Name as the active file, and is indicated by a P in the Set field.

Changing Your Autosampler's Setup

❖ To change the cursor speed

You can adjust the cursor speed and display contrast.

1. Press MENU and select **OPTIONS > Configurations**.
2. Move the cursor to the Key Repeat Rate field.
3. Use the + and − keys to select between **Slow**, **Medium**, and **Fast**.

❖ To change the display contrast

1. Press STATUS to access the Status Screen.
2. Press ▶.
3. Do one of the following:
   - To increase the contrast, use the + key.
   - To decrease the contrast, use the − key.

❖ To turn off the door interlock

When the door interlock is enabled, the XYZ arm moves to the back-left corner of the tray compartment when you open the door.

❖ To turn off the interlock

1. Press MENU and select **OPTIONS > Ready Participation**.
2. Move the cursor to the Door Interlock field and use the + and − keys to select **DISABLE**.

Quick Start: Starting Runs from the Command Center

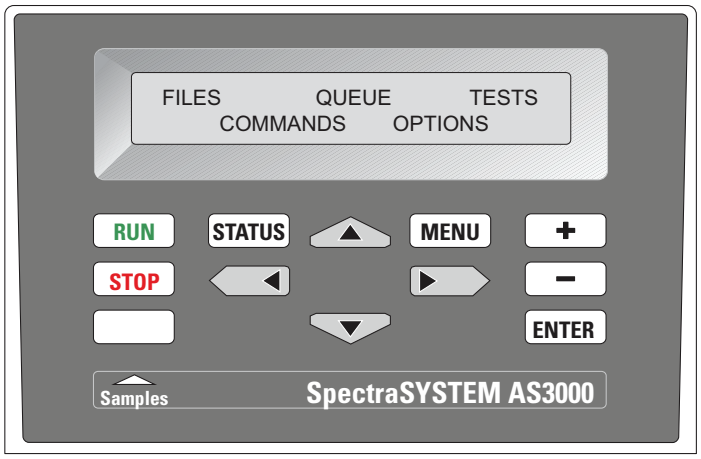
This guide describes how to start runs from the command center. If you have not set up your LC system, inject distilled water rather than an actual sample.

Contents

Command Center

For information on the menu screens, see "SpectraSYSTEM AS3000 Menus." For information on the keypad controls, see the "SpectraSYSTEM AS3000 Quick Reference Guide."

Figure 1. Command center



Loading Trays into the Autosampler

Trays A, B, and C are arranged from left to right in the sample tub. The position number is indicated on the top left of each vial position.

❖ **To install your trays**

1. Fill a vial with distilled water.
2. Place the vial in position 1 of tray A, location A01.
3. Hold the tray handle and tilt the back end down.
4. Insert the tray's runner into the slots at the back of the sample tub and lower the front of the tray into place.
5. Press down firmly to seat the tray and pull the handle back toward you to be sure the tray is properly seated.

Setting the Operation Mode to Stand Alone

❖ **To set the operation mode to Stand Alone**

1. Turn on your autosampler by pressing the on/off switch in the lower right corner of the autosampler.

   When the startup sequence is complete, the Status screen appears.

   Figure 2. Status screen

QStatus	Set	#Samples
IDLE	0	0/ 0

2. Press MENU and select **Options > Configurations**.
3. Press ▼ to move down the Configurations menu.
4. In the Mode field, select **Stand Alone**.

Starting a Run

To start a run, do one of the following:

- To customize the injection settings, follow these steps:
  - Step 1: Creating Your File
  - Step 2: Loading Your File
  - Step 3: Building a Sample Queue
  - Step 4: Starting Your Run
- If you want to use the default settings, go directly to these steps:
  - Step 3: Building a Sample Queue
  - Step 4: Starting Your Run

Step 1: Creating Your File

Create files when you want to use more than one set of injection parameters in the queue, when you want to store a specific set of injection parameters, or both.

The default file contains these parameter settings.

Menu parameters	Default setting
Injection	
Injection Volume	10 µL
Injections/Sample	1
Cycle Time	10.0 minutes
Column Oven	Off
Tray Temp	Off

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Menu parameters	Default setting
Calibration	
The default value in the Samples/Calibration field is zero, which means that the sample set contains no calibration vials and the autosampler ignores the other settings in the Calibration menu.	
Number of Levels	1
Samples/Calibration	0
Injections/Level	1
Injection Volume	10
Type	Reuse same vials
Bracket Samples	No
More	
Equilibration Time	0.00 minutes
Gradient Delay	0.00 mL
Sample Viscosity	Normal
Flush Volume	400 µL
Injection Type	Push Hi
Injection Range	1–200 µL
Needle Height	2 mm

❖ To create your file

1. Press **MENU** and select **FILES > Edit.**

   The Edit menu appears.

   Figure 3. Edit menu

   ```
   Edit File                1_
   File Name:               PRACTICE
   - - - - - - - - - - - - - - - - - -
   Injection
   Calibration
   - - - - - - - - - - - - - - - - - -
   Timed Events
   More
   ```

2. Press **ENTER** to edit File 1.
3. Use the use the ◀, ▶, **+**, and **−** keys to specify each character in the file name of your choice. For this example, enter the name **PRACTICE**.
4. Move the cursor to each field in the Injection, Calibration, Timed Events, and More menus and press **ENTER** to accept the default values.

> **Note** After the last field in each menu, the next most probable choice in the menu structure appears. For example, after you accept the default values in the Calibration menu, the screen returns to the Edit menu with the cursor in the Timed Events field. Similarly, after you accept the default values in the More menu, the screen returns to the File menu with the cursor in the Load field. This pattern occurs throughout the user interface. You can override the default choice at any time by moving the cursor where you like.

Step 2: Loading Your File

❖ To manually load your file

1. Select **Edit > Load**.
2. Verify that File 1 is entered, and then press **ENTER** to load it into the autosampler's active memory.

   The **File Loaded** message appears briefly, and then the Status screen appears (Figure 4). Loading the file activates the temperature control options that you set in the Injection menu.

   Figure 4. Queue Status screen

   ```
   QStatus     Set        #Samples
   IDLE         0          0/  0
   ```

Step 3: Building a Sample Queue

❖ To build a sample queue

1. Press the Samples key to display the Samples menu.

   If you have not loaded a file, the File field is blank.

   Figure 5. Samples menu

   ```
   Sample Set 1
   File 1:
   - - - - - - - - - - - - - - - - - -
   Injection Volume          10.0
   Injections/Sample         1
   - - - - - - - - - - - - - - - - - -
   Cycle Time                10.0
   First Sample Vial         A01
   - - - - - - - - - - - - - - - - - -
   Number of Samples         1
   Add to Queue              YES
   ```

2. To move down the Samples menu, press ▼.
3. To change the Cycle Time to **1.0**, use the ◀, ▶, **+**, and **−** keys.

   The cycle time is the time between injections. If you are injecting an actual sample, enter an appropriate cycle time for your application (injection time + data acquisition time).
4. When you reach the Add to Queue line, press **ENTER** to add the sample set (1) to the queue.

Step 4: Starting Your Run

❖ To start your run

1. If you have not already done so, place a vial filled with distilled water in position A01.
2. Press **RUN**.

   If everything is running properly, the QStatus changes from IDLE to RUN (Figure 6). The autosampler picks up the vial, carries it to the sample tower, injects the sample, returns the vial to the sample tray, and flushes the lines.

   Figure 6. Queue Status screen with the queue running

   ```
   QStatus     Set        #Samples
   RUN          1          1/  1
   ```

   The injection status field displays the time elapsed since the run started (Figure 7). When the run ends, the QStatus returns to IDLE.

   Figure 7. Injection Status screen with a run in progress

   ```
   Status      Vial       Vol        #Inj
   0.05        A01-S      10         1/  1
   ```

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