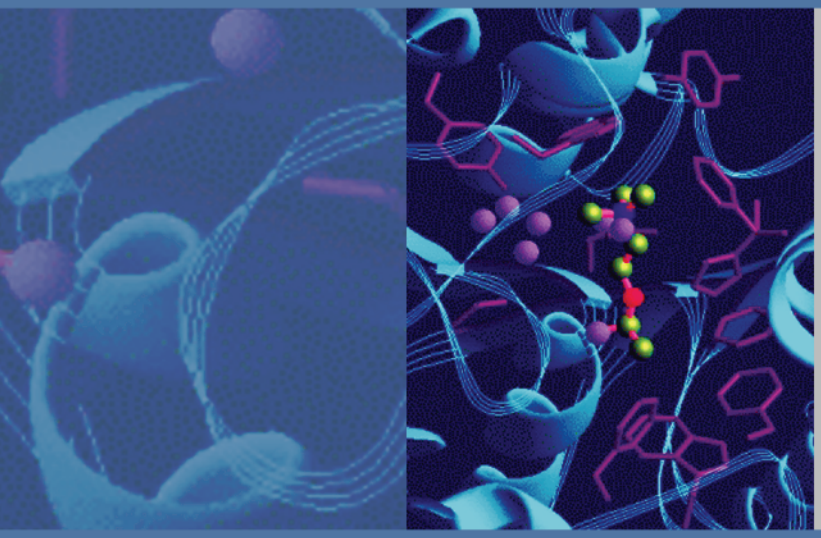


Micro AS Autosampler

Hardware Manual

97255-97020 Revision B February 2009

DOCUMENTATION SURVEY

Thermo SCIENTIFIC

Software Version: Xcalibur 2.0 SR2 through 2.0.7, LC Devices 2.0.2, 2.1.0, and 2.2.0

For Research Use Only. Not regulated for medical or veterinary diagnostic use by U.S. Federal Drug Administration or other competent authorities.

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.

EMC Directive 89/336/EEC. 92/31/EEC, 93/68/EEC

EMC compliance has been evaluated by KEMA (The Netherlands) and Underwriters Laboratory Inc. (USA).

EN 55011	1998	EN 61000-4-4	1995, 2001
EN 61000-3-2	1995	IEC 61000-4-4	2000, 2001
EN 61000-3-3	1991	EN 61000-4-5	1995, 2001
EN 61326-1	1997	IEC 61000-4-5	1995, 2000
EN 61000-4-2	1995, 1998, 2001	EN 61000-4-6	1996, 2001
IEC 61000-4-2	1995, 1998, 2000	IEC 61000-4-6	1996, 2000
EN 61000-4-3	2002	EN 61000-4-11	1994, 2001
IEC 61000-4-3	2002	IEC 61000-4-11	1994, 2002
IEC 61000-3-2	2000	CISPR11	1999
IEC 61000-3-3	1994		

CFR47, Part 15, Subpart B: 2003

Low Voltage Safety Compliance

Compliance with Safety Directives has been evaluated by UL International (The Netherhlands), authorizing the Micro As autosampler to carry both UL and cUL marking.

Safety Compliance Directives are 89/392/EEC, 91/368/EEC, 93/44/EEC, 73, 23, EEC, 93/68/EEC, and harmonized standard EN61010-1:1993, 1995 (safety requirements for laboratory equipment).

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 heavy and/or bulky for one person alone to handle 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

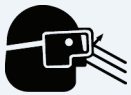
Dieses Produkt muss die EU Waste Electrical & Electronic Equipment (WEEE) Richtlinie 2002/96/EC erfüllen. Das Produkt ist durch folgendes Symbol gekennzeichnet:

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 corprelles. 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 elensver 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 chemiques dangereux peuven se trouver dans l'instrument. Proted dos gants pour manipuler tous produits chemiques 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:** Dex projections chimiques, liquides, ou solides peuvent être dangereuses pour les yeux. Porter des lunettes de protection lors de toute manipulationde 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'un 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 *Micro AS Autosampler Hardware Manual* provides you with information on how to install, operate, maintain, and troubleshoot the Thermo Micro AS autosampler.

Related Documentation

In addition to this guide, Thermo Fisher Scientific provides Help available from within the software.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

CAUTION Highlights hazards to humans, property, or the environment. Each CAUTION notice is accompanied by an appropriate CAUTION symbol.

IMPORTANT Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

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 mssupport.thermo.com and click **Customer Manuals** in the left margin of the window.

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- Complete a brief survey about this document by clicking 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

Welcome to the Thermo Micro AS autosampler. The Micro AS autosampler is a member of the Thermo Scientific family of LC devices.

The Micro AS autosampler offers a wide variety of capabilities for HPLC. It is designed for routine analysis and method development, and is fully compatible with a variety of plate types and with other laboratory equipment. The Micro AS features four injection methods: full loop injection, partial loopfill injection, and two types of µL pickup injections. (See "Injection Methods" on page 9 for more information.)

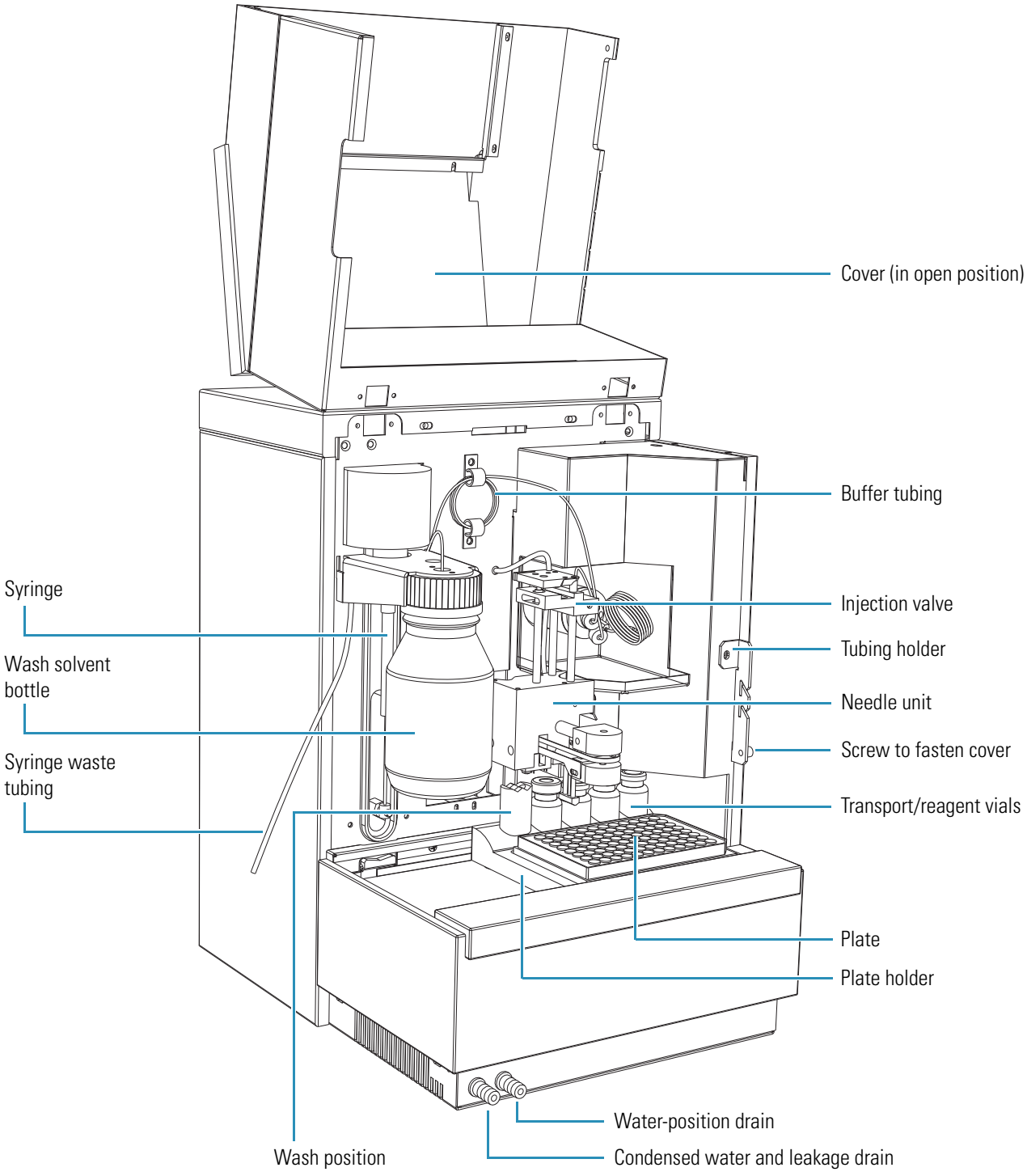
This chapter describes the parts of the Micro AS autosampler and their functions.

Contents

Autosampler Components

Figure 1 illustrates the components visible from the front view of the Micro AS autosampler.

Figure 1. Front view of the Micro AS autosampler



The major components of the autosampler are as follows:

- Injection System
- Plate Holder
- Back Panel
- Keypad and Display of the Micro AS

Injection System

The major components of the injection system are as follows:

- Syringe
- Needle Unit
- Buffer Tubing
- Injection Valve
- Sample Loop
- Wash Position

Syringe

The Micro AS autosampler is equipped with a 25 µL syringe.

Needle Unit

The needle used for sampling consists of two parts:

- A prepuncturing needle, which is a hollow needle used for puncturing of the septum, capmat or sealer covering the sample vials or wells. The prepuncturing needle can also be used to apply headspace pressure of approximately 0.5 bar (7.25 psi) to the sample.
- A sample needle, which is placed inside the hollow prepuncturing needle and used for the actual transport of sample.

Note Most commercially available sealers or capmats cannot be used in combination with headspace pressure. Switch off headspace pressure (in the General Menu) when using non-compatible sealers or capmats.

A sensor on the end of the needle assembly detects the presence or absence of plates or vials and automatically determines plate height.

Buffer Tubing

The buffer tubing connects the syringe to port 3 of the injection valve. The buffer tubing prevents contamination of the syringe by sample. (For more information on the role of the buffer tubing during injections, refer to "Injection Methods" on page 9.)

The standard buffer tubing supplied with the Micro AS autosampler has a volume of 50 µL.

Injection Valve

The Micro AS is equipped with a Valco C2-1346 injection valve. The valve controls the loading of sample onto the column through the sample loop. The valve supports four injection modes: full loop injection, partial loopfill injection, µL pick-up injection, and µL pick-up (qualitative analysis) injection. For more information on these injection methods and the operation of the valve, refer to "Injection Methods" on page 9.

Sample Loop

The sample loop is a length of PEEK™ tubing connected between port 2 and port 5 of the injection valve. During an injection, the sample is drawn into the sample loop when the injection valve is in the Load position, and pushed onto the column when the injection valve is in the Inject position.

The Micro AS is equipped with a 20 µL sample loop. Depending on the volume of sample you want to inject, you might need to use a smaller sample loop. Refer to "Choosing the Sample Loop" on page 46 for information on choosing the correct size of sample loop for your application.

Wash Position

The wash position is the home position of the needle assembly. The needle returns to this position between runs or when a wash step is programmed. During a wash, the white plastic receptacle collects the wash liquid and channels it to the waste outlet.

Plate Holder

The Micro AS accommodates the following types of plates:

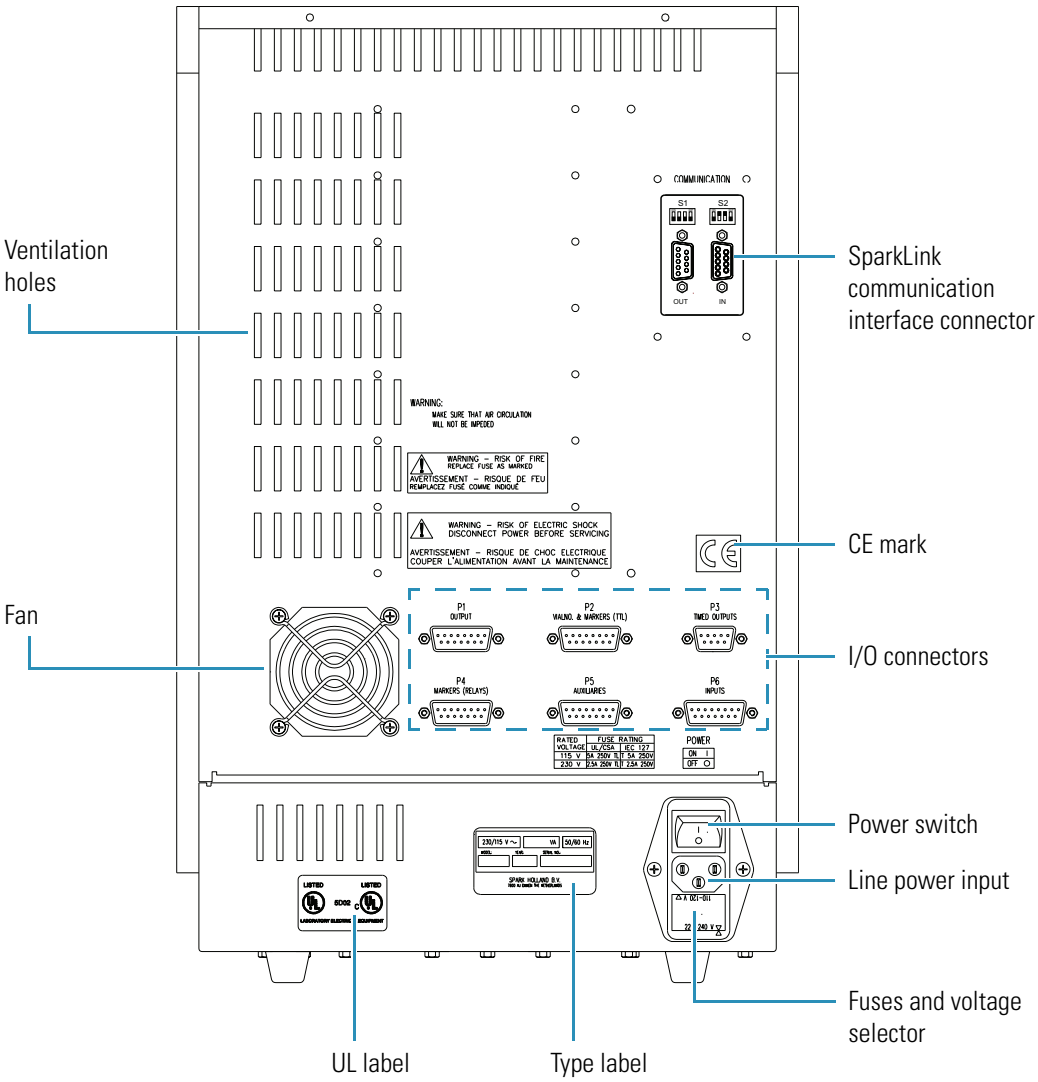
- 96 low wells
- 96 high wells
- 384 low wells
- 48 vials

The back of the plate holder contains four slots that can hold 10 mL (22 mm × 47 mm) vials. These vials can be used to hold either reagents to be mixed with samples prior to injection, or transport liquid used to transport the sample into the sample loop during µL pick-up injections.

Back Panel

Figure 2 illustrates the back panel of the Micro AS autosampler.

Figure 2. Back of the Micro AS autosampler



The back panel of the autosampler contains the following major components:

- Communications and I/O Connectors
- Power Switch and Line Power Input
- Fuses and Voltage Selector

Communications and I/O Connectors

The Micro AS autosampler has two nine-pin connectors located in the upper right area of the back panel. See Figure 2. The right-hand connector (marked 'IN') is used to connect the Micro AS to the data system computer. The left-hand connector (marked 'OUT') is not used with the Xcalibur™ data system.

Six I/O connectors (5 output connectors and one input connector) are located on the back panel of the Micro AS. See Figure 2. The output connectors can be used to trigger external devices from the Micro AS. The input connector allows signals from an external device to trigger actions by the Micro AS autosampler.

For more information on the configuration of the I/O connectors, refer to Chapter 3, "I/O Connections."

Power Switch and Line Power Input

The power switch is used to turn power to the autosampler on and off. The autosampler power cord plugs into the line power input. The Micro AS autosampler can operate using either 115 V ac ± 10% or 230 V ac ± 10%. Make sure that the voltage selector is set correctly and the correct fuses are installed for the voltage at which you intend to operate the autosampler. For more information on the voltage selector and fuses, refer to "Fuses and Voltage Selector" on page 6.

Fuses and Voltage Selector

Two fuses are located behind a covering plate beneath the line power input. These fuses protect the autosampler circuitry from current overloads. The type of fuse required depends on your operating voltage. The correct fuses for your operating voltage are installed in the autosampler before it is shipped to you. The required fuse types are:

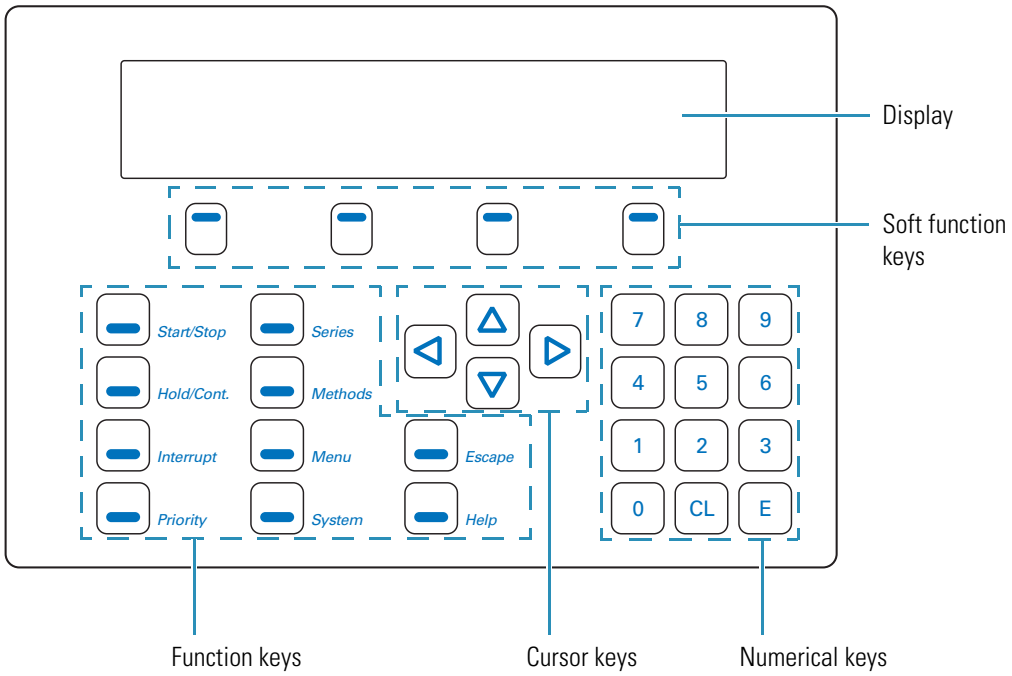
- 115 V ac: Two 5.0 AT-fuses (0.25 in. × 1.25 in., UL/CSA)
- 230 V ac: Two 2.5 AT-fuses (5 mm × 20 mm, IEC 127)

The fuse cover plate also indicates the current operating voltage. The current operating voltage is the one displayed right-side up at the bottom of the plate. (The voltage not currently in use is printed upside down at the top of the plate.) If you want to change the operating voltage, you need to remove the plate, change the fuses to the appropriate ones for the new voltage, and reinstall the plate in the opposite orientation. For more information on changing fuses, refer to "Replacing Fuses" on page 83.

Keypad and Display of the Micro AS

Under most circumstances, you control the Micro AS autosampler using the Xcalibur software installed on your data system computer. Refer to Chapter 6, "Controlling the Micro AS from Xcalibur," and the *online Help* for more information on this software. You can also operate and program the Micro AS using the front keypad and display. Figure 3 illustrates the keypad and display of the Micro AS.

Figure 3. Keypad and display of Micro AS Autosampler



The functions of the keys are as follows:

- Soft function keys

  The label assigned to these keys depends on the menu that is active. The label of each key is shown in the bottom line of the display.

- Cursor keys

  The keys can be used to move to a different field in the display, to move to a different field in a menu, or to make a displayed value higher or lower.

- Numeric keys

  – 0 to 9: To enter numerals in the various programming fields.

  – CL: To clear a value in a field or replace it by NONE or AUTO.

  – E stands for Enter: To go through menu lines or to confirm a choice made in a menu or a value entered. The entered value is checked for validity and then saved.

- Function keys
  - Run control keys
    - Start/Stop: To start or stop automatic processing, or to reset the system after an error has occurred.
    - Hold/Cont.: To hold or continue the analysis time. The analysis time is extended by the period that Hold is active.
    - Interrupt: Not used
    - Priority: To stop a run to process a priority sample before analyzing the rest of the programmed sample series. Before the run is interrupted processing of the present sample will be finished. As soon as the priority sample has been analyzed, the analytical run is resumed. A priority sample is a series of one well with an injection method, a wash method and a time base method defined in a template. Priority samples may only be run if the correct settings have been entered in the System Menu.
  - Programming keys

    (Refer to Appendix A, "Introduction to Keypad Operation," and Appendix B, "Keypad Menu Reference," for more information.)
    - Series: To enter the Series Menu in which series can be defined for an analytical run.
    - Methods: To enter the Methods Menu in which methods can be programmed for use in an analytical run.
    - Menu: This key can only be used if MENU or MN is shown in the top right hand corner of the display. Press this key to display additional menu choices.
    - System: To enter the System Menu in which system settings can be entered.
  - General keys
    - Escape: Allows the user to leave the programming mode or go to a previous level in the menu. Entered values are checked for validity and then saved.
    - Help: To display help information. Help is available only for a limited number of functions.

Injection Methods

This section describes the injection methods offered by the Micro AS autosampler. The autosampler offers four injection methods:

- Full Loop Injection
- Partial Loopfill Injection
- µL Pick-up
- µL Pick-up (Qualitative Analysis)

Note The µL pick-up (qualitative analysis) injection mode is available only when you control the Micro AS from your data system computer using the instrument control software. This injection mode cannot be programmed from the autosampler front keypad.

Table 1 summarizes the features of the four injection methods.

Table 1. Micro AS autosampler injection methods (Sheet 1 of 2)

	Full loop	Partial loopfill	µL pick-up	µL pick-up (qualitative analysis)
Description	The sample loop is completely filled.	The sample loop is partially filled.	A small volume of sample is transported into the sample loop by aspiration of transport liquid (mobile phase)	A small volume of sample is transported into the sample loop by aspiration of transport liquid (mobile phase)
Permitted injection volume	Sample loop volume	0.01 µL up to 50% of sample loop volume	0.01 µL up to (sample loop volume - 3 × needle volume)/2	Sample loop volume
Sample Loss	Sample loss = 2 × loop volume + flush volume	Sample loss = flush volume	No sample loss	No sample loss

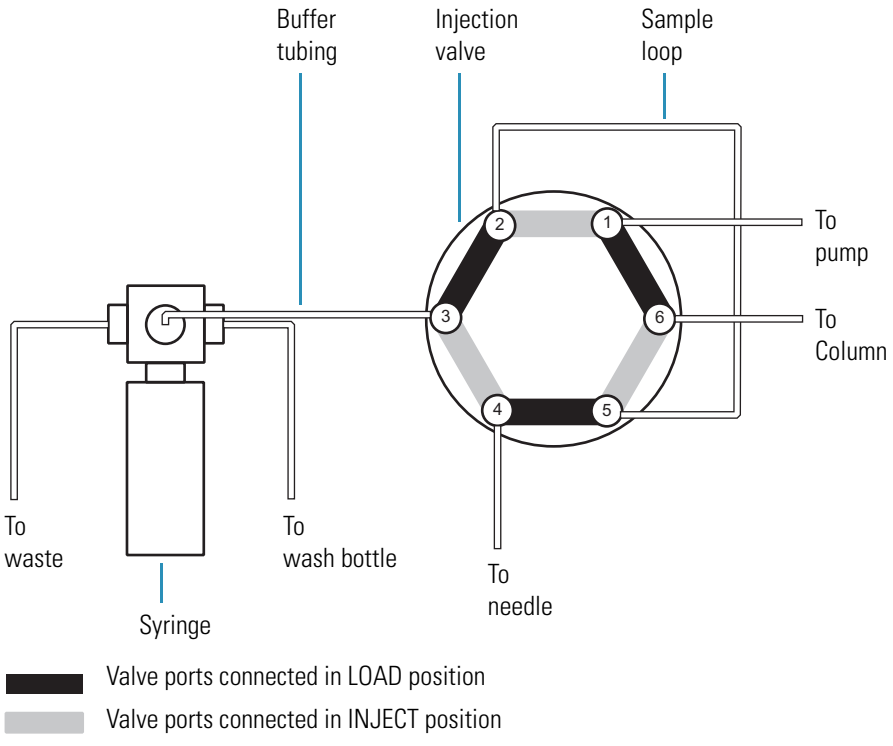
Table 1. Micro AS autosampler injection methods (Sheet 2 of 2)

	Full loop	Partial loopfill	µL pick-up	µL pick-up (qualitative analysis)
Reproducibility	Maximum reproducibility. Relative Standard Deviation (RSD) < 0.3%	RSD < 0.5%	RSD < 1%	Not determined
Accuracy	±10% (accuracy of sample loop volume)	Maximum accuracy. Accuracy depends on syringe accuracy.	Maximum accuracy. Accuracy depends on syringe accuracy.	Maximum accuracy. Accuracy depends on syringe accuracy.

The Micro AS uses a syringe to aspirate sample from a well into the sample loop. To prevent contamination of the syringe the Micro AS is equipped with a buffer tubing between the syringe and the injection valve. Wash solvent is used to remove sample from the buffer tubing and sample needle, and to rinse the buffer tubing and sample needle.

For an overview of the fluid connections of the Micro AS, see Figure 4.

Figure 4. Fluid connections of the Micro AS autosampler



Full Loop Injection

In a full loop injection, the syringe aspirates sufficient sample to overfill the sample loop. The overfilling of the sample loop ensures that it is completely filled with pure sample. The full contents of the loop are expelled onto the column when the sample is injected.

Because the sample loop volume determines the injection volume, full loop injections are highly reproducible. However, because the volume of the sample loop is only specified to within 10%, they are less accurate than injections made by other methods. The injection volume cannot be varied without changing the sample loop. Full loop injections also require relatively large sample losses: the sample lost is equal to the loop overfill volume plus the flush volume. (Refer to Table 1 for more details.)

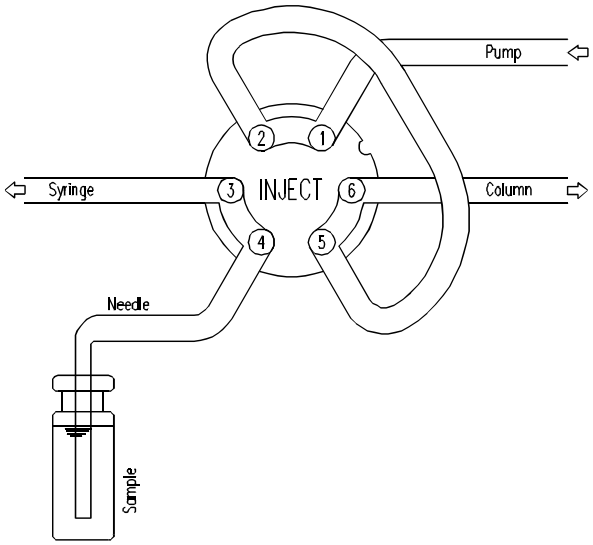
Use the full loop injection method if:

- You have relatively large amounts of sample.
- You need maximum reproducibility.
- You do not need to vary the injection volume over the course of a sequence run.

The switching sequence for a full loop injection is as follows:

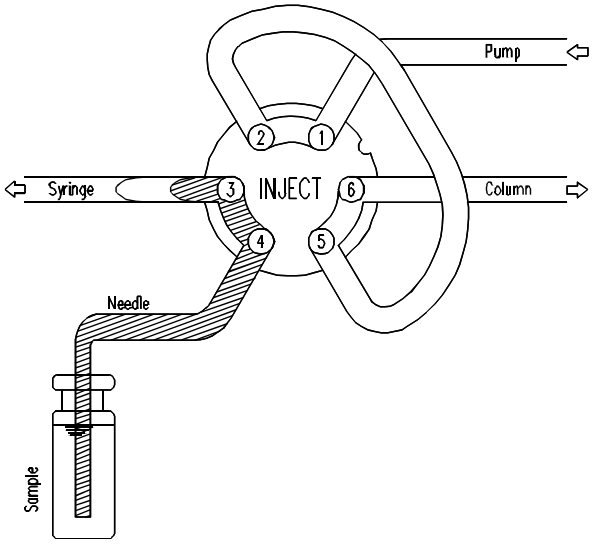
1. The injector starts in the INJECT position. The sample needle enters the well after the air needle prepunctures the septum. Headspace pressure is applied through the outer air needle to ensure that no air or vapor bubbles are formed during sample aspiration.

Figure 5. Full loop injection: injection valve in the initial state



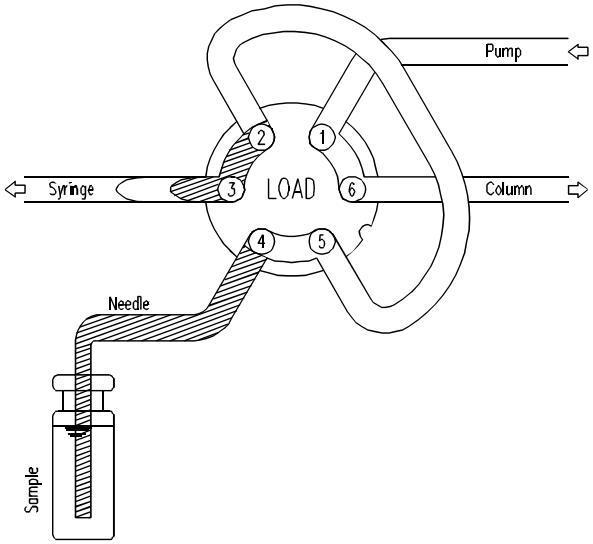
2. The syringe dispenser aspirates the programmed flush volume from the sample well to fill the sample line with sample and remove wash solvents.

Figure 6. Full loop injection: injection valve after aspiration of flush volume



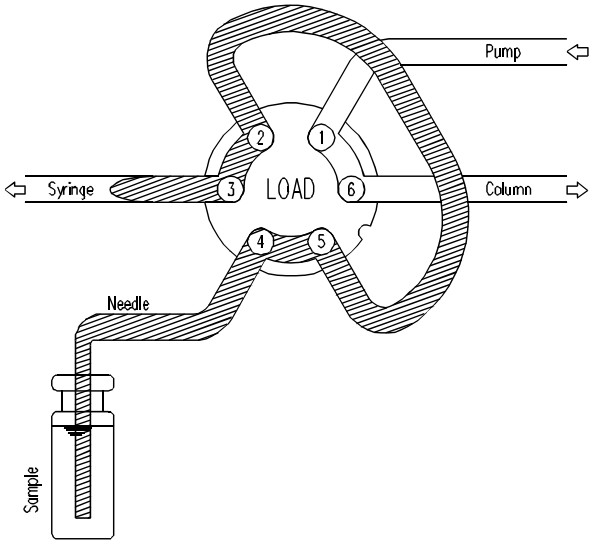
3. The injection valve switches to the LOAD position, placing a "sharp" sample front at the inlet of the sample loop.

Figure 7. Full loop injection: injection valve in the LOAD position and ready to draw sample from the vial



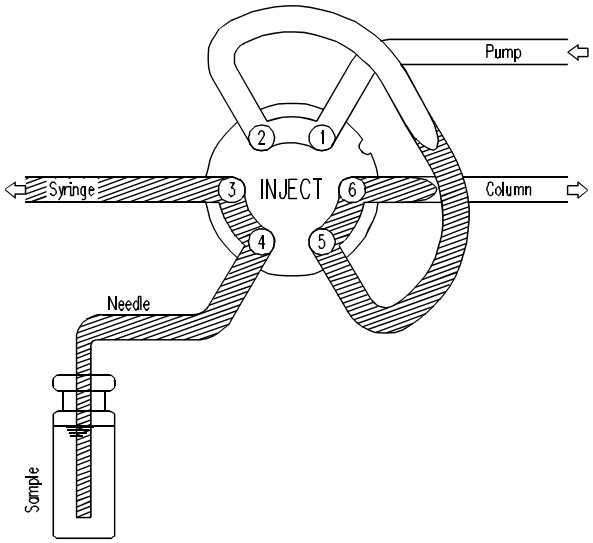
4. For full loop injections the sample loop is quantitatively filled by transporting three times the loop volume through the loop.

Figure 8. Full loop injection: injection valve after the sample loop has been filled



5. The injection valve switches to the INJECT position. The sample loop is now part of the HPLC mobile phase flow path; sample is transported to the column. The analysis time starts.

Figure 9. Full loop injection: injection valve as sample is injected onto the column



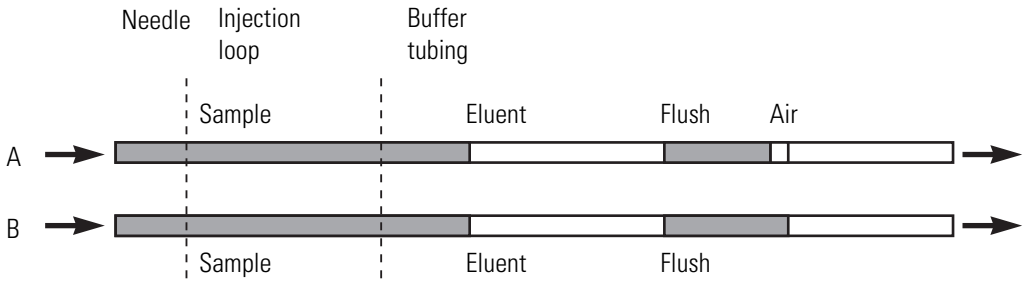
If only one injection is to be done from each well, or if a wash routine has been programmed to execute after every injection, the needle withdraws from the well immediately after the injection. If a wash is programmed, the autosampler immediately performs a wash. After the analysis time has passed, a new sequence is started.

If more than one injection is done from the same well without a wash between injections, the Micro AS withdraws a flush volume after the analysis time is complete to compensate for diffusion of mobile phase from the rotor groove into the first part of the sample line during the analysis time. The flush volume between injections is always 50% of the programmed flush volume. If the total amount of sample withdrawn with the next injection from the well will exceed the total volume of the buffer tubing, the buffer tubing is emptied into the wash position before the next injection. The next fill sequence then starts with a full flush volume.

An air segment can be used to reduce the amount of flush volume (see Figure 10). During a full loop injection, the air segment is aspirated in front of the flush volume. The air segment is not injected onto the column and does not influence the injection. Use of an air segment can be enabled in the General Menu.

With the default 2.4 µL needle, the minimum programmable flush volume is 0 µL for injections. You might experience decreased performance if you use a flush volume of less than twice the needle volume. If your samples are highly viscous, you might need to program larger flush volumes and reduce the syringe speed for better performance.

Figure 10. Sample path for full loop injection, with and without air segment



A = With air segment
B = Without air segment

Partial Loopfill Injection

In a partial loopfill injection, the sample loop is not filled completely. Only the desired injection volume of sample, which must be equal to or less than 50% of the sample loop volume, is drawn into the loop.

Partial loopfill injections allow the injection volume to be varied over the course of a sequence run. They also require less sample loss than full loop injections: sample loss is equal to the flush volume. Partial loopfill injections offer greater accuracy than full loop injections, but decreased reproducibility.

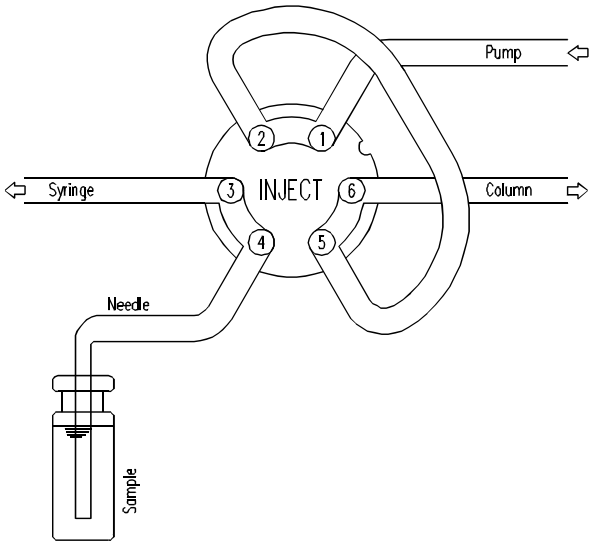
Use the partial loopfill injection method if:

- You need to vary injection volumes over the course of a sequence run.
- You need maximum accuracy.

The switching sequence for a partial loopfill injection is as follows:

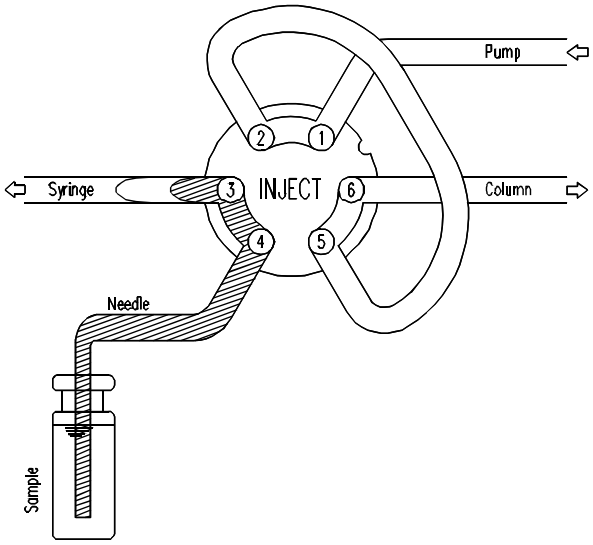
1. The injector starts in the INJECT position. The sample needle enters the well after the air needle prepunctures the septum. Headspace pressure is applied through the outer air needle to ensure that no air or vapor bubbles are formed during sample aspiration.

   Figure 11. Partial loopfill injection: injection valve in the initial state



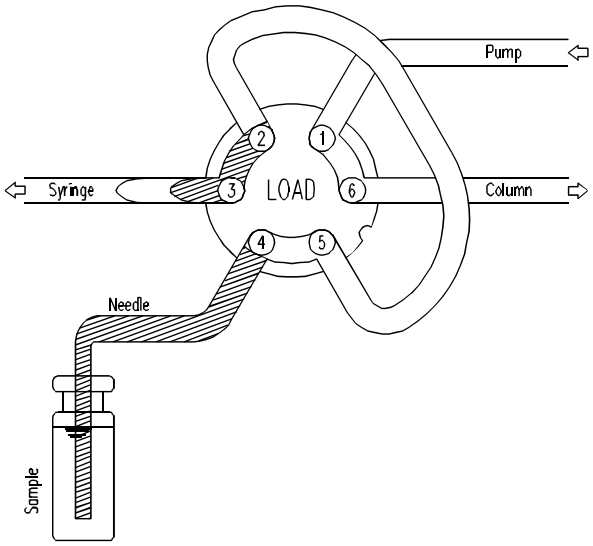
2. The syringe dispenser aspirates the programmed flush volume from the sample well to fill the sample line with sample and remove wash solvents.

   Figure 12. Partial loopfill injection: injection valve after aspiration of flush volume



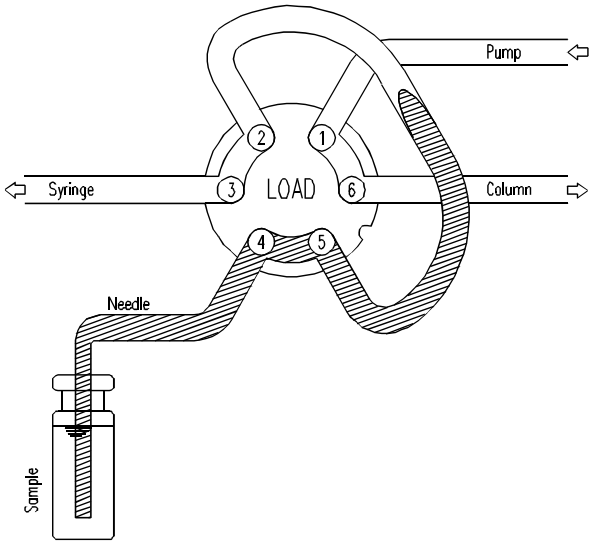
3. The injection valve switches to the LOAD position, placing a "sharp" sample front at the inlet of the sample loop.

Figure 13. Partial loopfill injection: injection valve in the LOAD position and ready to draw sample from the vial



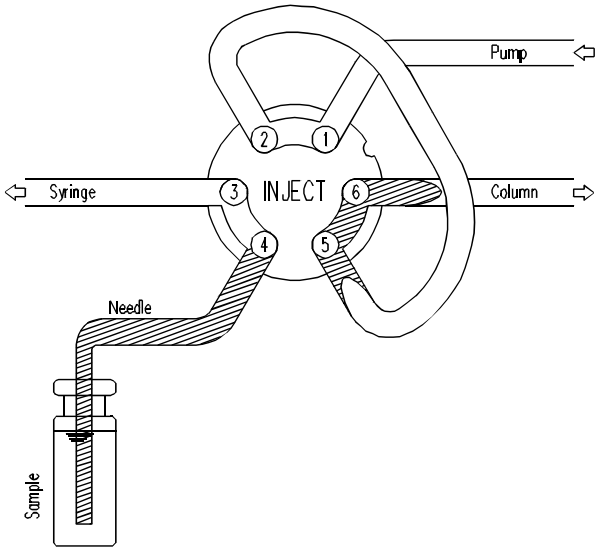
4. For partial loopfill injections the sample loop is filled by transporting the programmed injection volume into the sample loop.

Figure 14. Partial loopfill injection: injection valve as sample is drawn into the loop



5. The injection valve switches into the INJECT position. The sample loop is now part of the HPLC mobile phase flow path; the sample is transported to the column. The analysis time starts.

Figure 15. Partial loopfill injection: injection valve as sample is injected onto the column

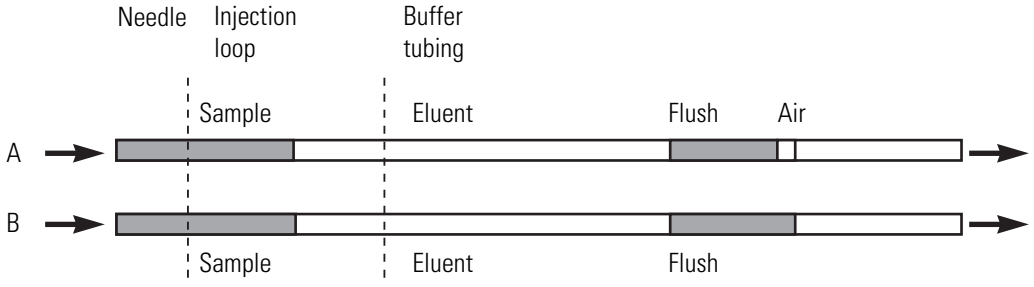


If the next injection is to be performed from the same well, and no wash step is programmed, the next injection sequence begins with a flush of half the programmed flush volume. Otherwise, the next injection sequence begins with a flush of the programmed flush volume.

Note Use of a flush volume of less than twice the volume of the needle might result in decreased performance.

An air segment can be used to reduce the amount of flush volume (see Figure 16). This air segment is at the front of the flush volume and is not injected. Use of an air segment can be enabled in the General Menu.

Figure 16. Sample path for partial loopfill injection, with and without air segment



A = With air segment
B = Without air segment

µL Pick-up

In a µL pick-up injection, the syringe aspirates a very small amount of sample and transports it into the sample loop by aspirating an appropriate volume of transport liquid. The transport liquid should be the same as the mobile phase, to avoid disturbing the chromatogram with extra solvent peaks.

µL pick-up injections allow injections of very small volumes with no sample loss. They offer accuracy equal to that of partial loopfill injections, but have the lowest reproducibility of the three available injection methods.

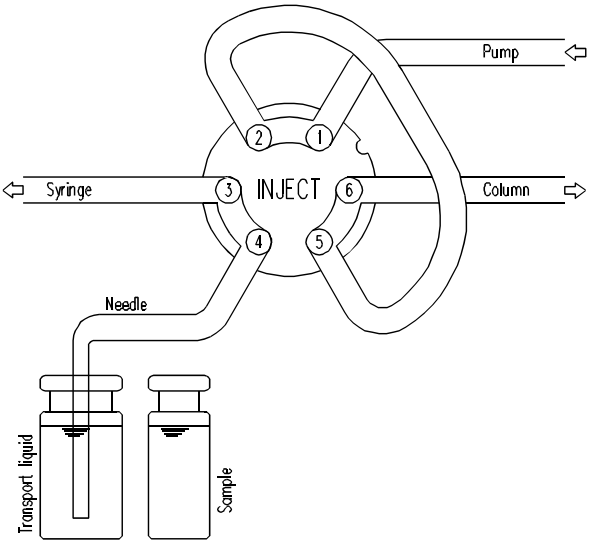
Use the µL pick-up injection method if:

- You have a relatively small amount of sample and need to minimize sample loss.
- You need to inject very small volumes.

The switching sequence for a µL pick-up injection is as follows:

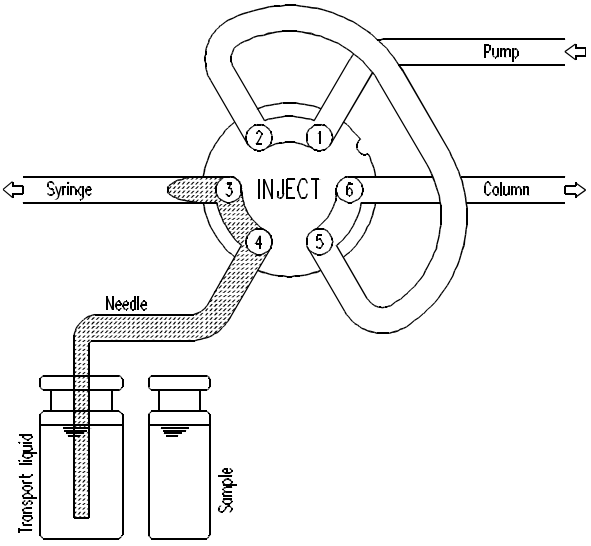
1. The injection valve starts in the INJECT position. The sample needle enters the vial of transport liquid (mobile phase, to avoid disturbance of the chromatogram with an additional peak of the transport solvent) after the air needle prepunctures the septum. The headspace pressure is applied through the outer air needle to ensure that no air or vapor bubbles are formed during solvent aspiration.

Figure 17. µL pick-up: injection valve in initial state



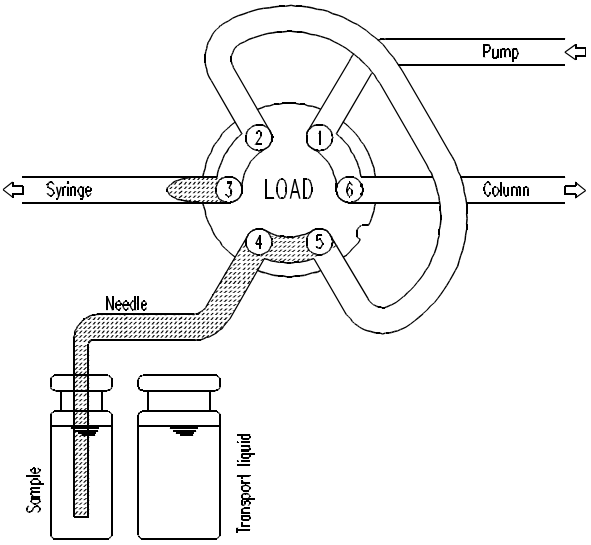
2. For the first injection after a wash or after emptying of the buffer tubing, the syringe dispenser aspirates transport liquid from the transport vial to fill the sample line with transport liquid and remove wash solvent.

Figure 18. µL pick-up: injection valve after initial aspiration of transport liquid



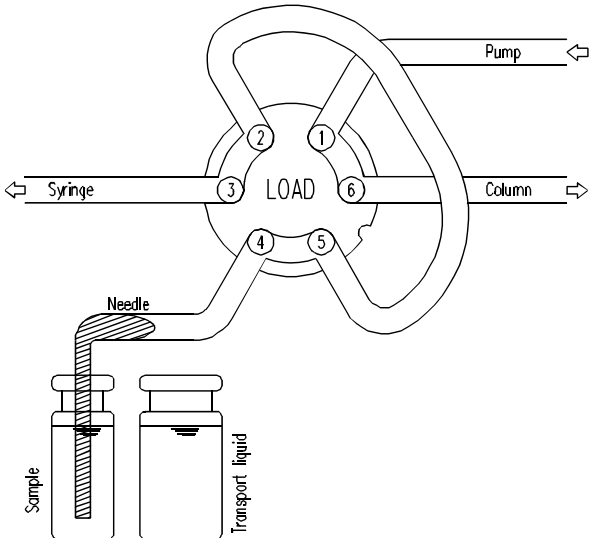
3. The needle moves from the transport vial to the sample well. The injection valve is switched to the LOAD position.

Figure 19. µL pick-up: injection valve ready to aspirate sample



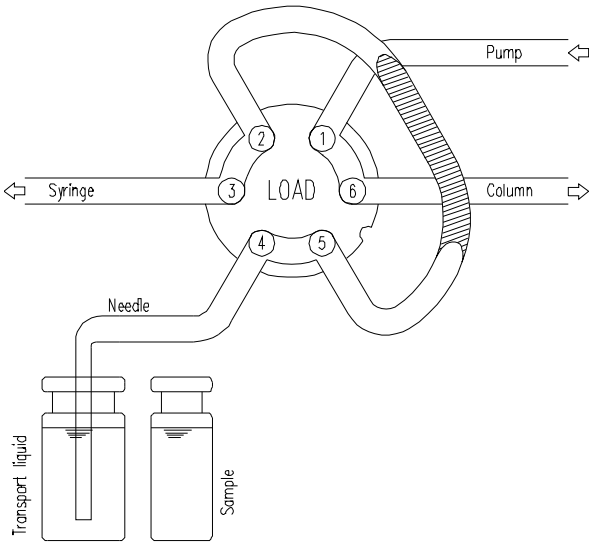
4. The programmed injection volume is aspirated from the sample well.

Figure 20. µL pick-up: injection valve after sample has been aspirated



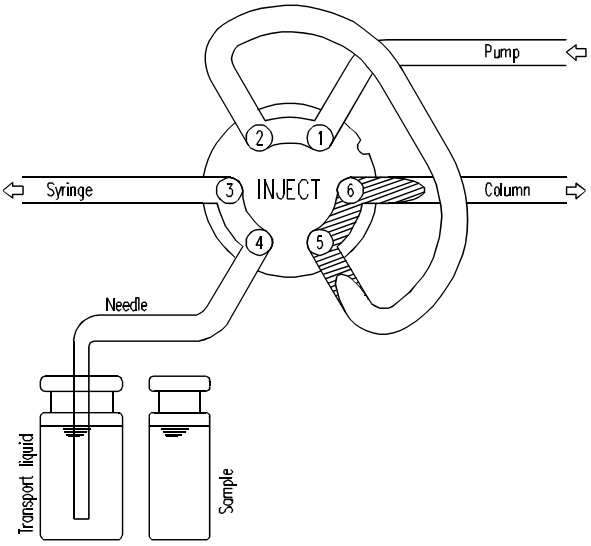
5. The sample needle moves back to the transport vial. The sample is quantitatively transported into the loop, with transport liquid (mobile phase) from the transport vial.

Figure 21. µL pick-up: injection valve after sample has been transported into loop



6. The injection valve is switched to INJECT. The sample loop is now part of the HPLC mobile phase flow path: sample is transported to the column. The analysis time starts to run.

Figure 22. µL pick-up: injection valve as sample is injected

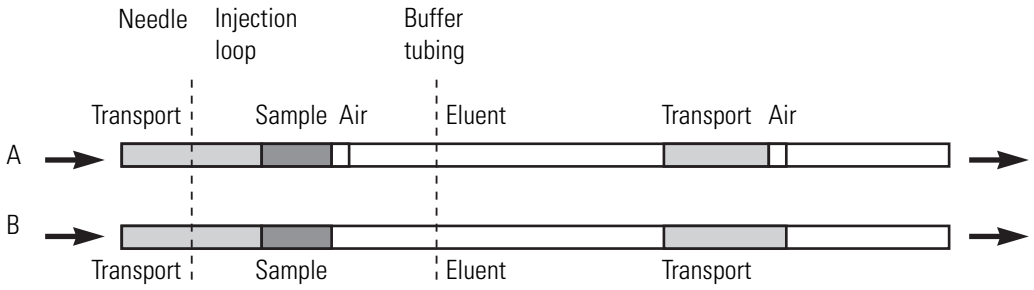


The next sequence skips the first withdrawal of transport solvent (step 2), unless a wash routine is performed or the Micro AS has emptied the buffer tubing to waste. In those cases the sequence is completely repeated.

If an air segment (see Figure 23) has been programmed, it is aspirated at the front of the first plug of transport liquid and at the front of every sample plug. Use of an air segment can be enabled in the General Menu.

Note If the air segment is enabled during a µL pick-up injection, the air segment at the front of the sample plug is injected into the HPLC system. It is recommended that you switch off the air segment option when using the µL pick-up injection method.

Figure 23. Sample path for µL pick-up injection, with and without air segment



A = With air segment
B = Without air segment

Note During µL pick-up injections, headspace pressure is not applied to sample wells to prevent errors due to air expansion when switching from the sample well to the transport vial.

µL Pick-up (Qualitative Analysis)

The µL pick-up (qualitative analysis) injection mode is available only when you control the Micro AS from your data system computer using the instrument control software. This injection mode cannot be programmed from the autosampler front keypad.

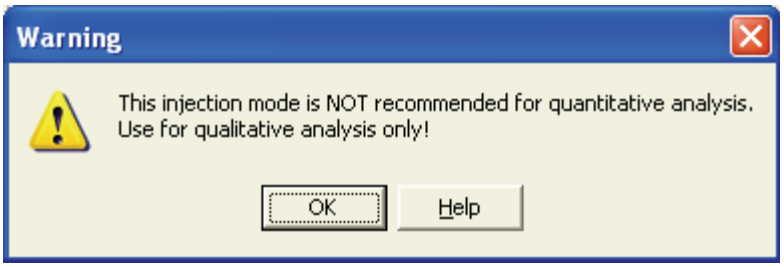
The operation of the autosampler valve during the µL pick-up (qualitative analysis) injection mode is exactly the same as during the ordinary µL pick-up injection. (Refer to "µL Pick-up" on page 18.) However, the µL pick-up (qualitative analysis) injection mode has less stringent injection volume limits. You can inject up to the full volume of the sample loop in this mode. Injections of volumes greater than [(sample loop volume - 3 × needle volume)/2] will have reduced reproducibility. Because of the reduced reproducibility, it is recommended that you use this injection method only for qualitative analysis, and not for applications in which precise quantitation is critical.

Use the µL pick-up (qualitative analysis) injection mode if:

- You want to minimize sample loss.
- You need to inject larger volumes than those allowed for by the µL pick-up injection mode.

When you use the Xcalibur Instrument Setup page to create and save an instrument method for the Micro AS using the µL pick-up (qualitative analysis) injection mode, you will see the warning message shown in Figure 24. Click **OK** to dismiss the warning message and save the method.

Figure 24. Warning message for µL pick-up (qualitative analysis) injection mode



Installation

This chapter describes how to install your Micro AS autosampler. The Micro AS shipping container contains a packing list. Please check that all items mentioned in the list are included in the package before you start the installation procedure for the Micro AS.

CAUTION Do not install the Micro AS in areas subject to shock, dust, or in direct sunlight. Do not place it near a source of heat, as this will disturb tray cooling.

CAUTION The Micro AS autosampler must only be connected to power sources and apparatus with protective grounding.

Contents

Installing the Micro AS

❖ **To install the Micro AS**

1. Lift the Micro AS from the shipping container. Make sure that you keep the autosampler upright by placing your hands under it.
2. Place the Micro AS in its operating location, preferably on the left hand side of the mass spectrometer as shown in Figure 32 on page 36. Make sure that the ventilation holes are not obstructed. Allow the instrument to acclimate for 1 hour.
3. Install the plate holder (see Figure 1) in the Micro AS. Place the plate holder on the flat surface underneath the wash solvent bottle, as far to the left and to the back as possible.
4. Check that the local line voltage matches the voltage indicated on the back panel of the Micro AS.

5. Connect the power cord to the Micro AS. (See Figure 2.) Plug the power cord into line voltage.
6. Switch on the Micro AS using the switch at the back of the apparatus. (See Figure 2, number 4.)
7. Using a Phillips screwdriver, remove the cover-fastening screw from the right side of the cover. (See Figure 1.)

The Micro AS starts up. The display indicates that a self-test and initialization procedure is being executed. After completion of this procedure, the following appears on the display:

The autosampler starts up in serial mode, ready for control by the computer data system.

Changing the Operation Mode

By default, the Micro AS autosampler starts up in the serial mode. If you want to operate the Micro AS autosampler from the Xcalibur data system, leave the autosampler in the serial mode. If you want to use the front panel controls, you must exit the serial mode.

This section contains the following topics:

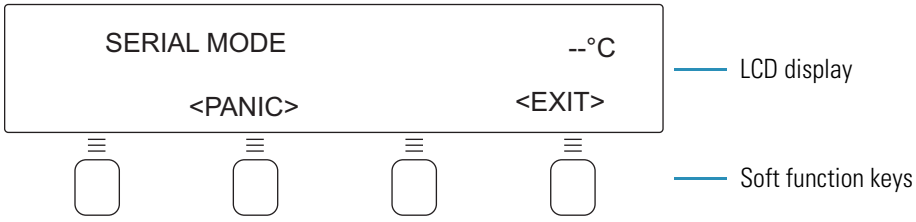
- Placing the Autosampler Under Front Panel Control
- Returning the Autosampler to the Serial Mode

Placing the Autosampler Under Front Panel Control

❖ **To operate the autosampler from the front panel controls**

Press the EXIT soft function key on the front panel as shown in Figure 25.

Figure 25. View of the Exit soft function key available from the serial mode



The Ready Menu appears. See Figure 26.

Note The autosampler must be placed into serial mode before you attempt to operate the autosampler using the data system control software. The data system computer will not be able to establish communication with the autosampler unless the autosampler is in serial mode.

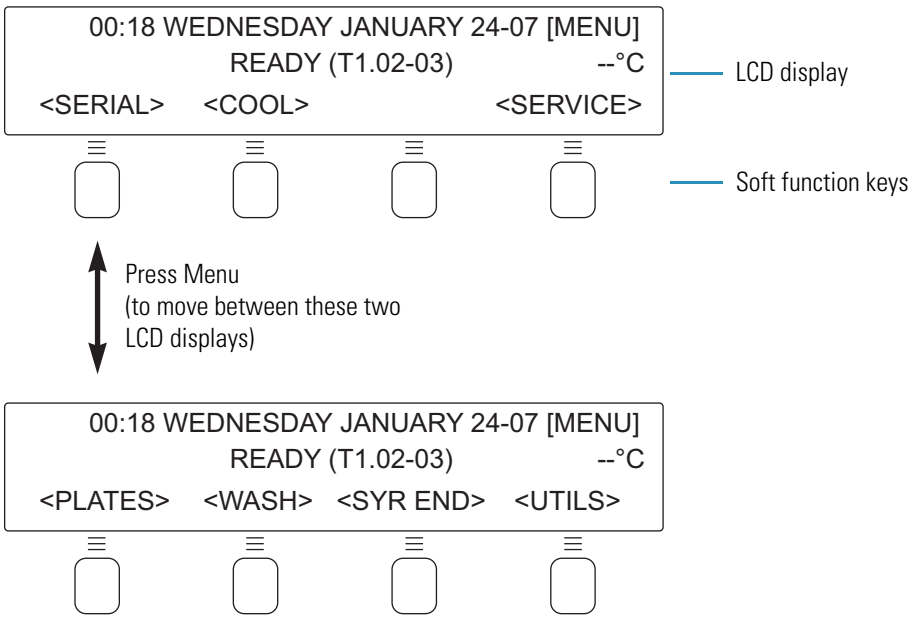
Returning the Autosampler to the Serial Mode

If the autosampler is under front panel control and you want to operate it from the Xcalibur data system, you must return it to the serial mode.

❖ To return the autosampler to the serial mode

1. Return to the Ready Menu shown in Figure 26.

   Figure 26. Ready Menu LCD display



2. Press the SERIAL soft function key.

   The autosampler returns to the serial mode.

Factory Installed Items

The Micro AS is factory installed with the following:

- Fuses (All fuses are UL-listed and CSA-certified):
  - 115 V ac ± 10%: two 5 AT fuses (slow, ¼ in. × 1¼ in., UL/CSA), or
  - 230 V ac ± 10%: two 2.5 AT fuses (slow, 5 × 20 mm, IEC127).
- 20 µL sample loop
- 25 µL syringe
- 50 µL buffer tubing
- Fused silica 2.4 µL sample needle

Table 2 gives the specifications for the standard tubings installed on the Micro AS autosampler.

For installation of HPLC connections, waste tubing, wash solvent, syringe, sample needle, plates, and so on, refer to Chapter 5, "Preparing for Use."

Table 2. Tubing specifications

Tubing	Material and dimensions
Standard sample needle and tubing	Fused silica tubing; 300 mm × 0.375 mm OD × 0.100 mm ID (total volume 2.4 µL)
Buffer tubing from high pressure valve to syringe valve	PEEK tubing; 260 mm × 1/16 in. OD × 0.50 mm ID (volume 50 µL)
Tubing syringe valve to wash solvent bottle	PTFE tubing; 260 mm × 1/16 in. OD × 0.50 mm ID
Tubing syringe valve to waste	PTFE tubing; 350 mm × 1/8 in. OD × 1/16 in. ID

I/O Connections

The Micro AS has six standard I/O connectors on the rear panel that can be used to allow the autosampler to control external devices or be controlled by an external device. There are five OUTPUT connectors and one INPUT connector. In addition, the autosampler has two 9-pin communications connectors, one of which is used to connect the autosampler to the data system computer. See Figure 2 for the location of the connectors.

Contents

Contact Closure Outputs

On the back panel of the Micro AS, the programmable output connector (labeled P1), marker output connector (labeled P4), and auxiliary output connector (labeled P5) are contact closure outputs (floating NO/NC contact). See Figure 27. Table 3, Table 4, and Table 5 give the configurations for these three connectors. The maximum voltage for these connectors is 28 V(dc or ac). The maximum current is 0.25 A.

Note Maximum current for 24 V_{DC} supply is 0.5 A total.

Table 3. Connector P1 OUTPUTS (2 programmable outputs and alarm output) (Sheet 1 of 2)

Output	Description
1	OUT 1 - Normally open
2	OUT 1 - Common
3	OUT 1 - Normally closed
4	OUT 2 - Normally open
5	OUT 2 - Common
6	OUT 2 - Normally closed

Table 3. Connector P1 OUTPUTS (2 programmable outputs and alarm output) (Sheet 2 of 2)

Output	Description
7	Spare
8	Spare
9	Spare
10	Alarm output - Normally open
11	Alarm output - Common
12	Alarm output - Normally closed
13	24 V dc
14	Power ground
15	Power ground

Note The Alarm output is activated whenever an error occurs. Refer to "Error Codes" on page 85 for a description of the error codes of the Micro AS.

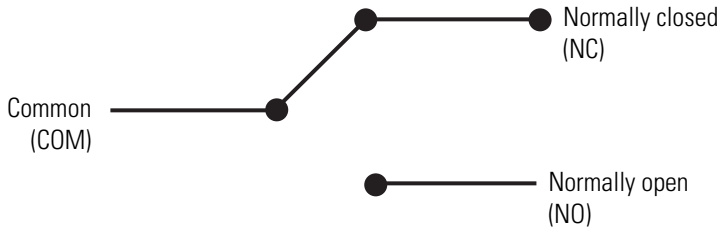
Table 4. Connector P4 MARKERS

Output	Description
1	Inject marker - Normally open
2	Inject marker - Common
3	Inject marker - Normally closed
4	Well marker - Normally open
5	Well marker - Common
6	Well marker - Normally closed
7	Labeled well marker - Normally open
8	Labeled well marker - Common
9	Labeled well marker - Normally closed
10	STOP I/O - Normally open
11	STOP I/O - Common
12	STOP I/O - Normally closed
13	24 V dc
14	Power ground
15	Power ground

Table 5. Connector P5 AUXILIARIES

Output	Description
1	AUX 1 - Normally open
2	AUX 1 - Common
3	AUX 1 - Normally closed
4	AUX 2 - Normally open
5	AUX 2 - Common
6	AUX 2 - Normally closed
7	AUX 3 - Normally open
8	AUX 3 - Common
9	AUX 3 - Normally closed
10	AUX 4 - Normally open
11	AUX 4 - Common
12	AUX 4 - Normally closed
13	24 V dc
14	Power ground
15	Power ground

Figure 27. Contact closure output



TTL Outputs

The TTL marker output connector (P2) and the 4 timebase code output connector (P3) are both TTL levels outputs. See Figure 28. The timebase code output can be programmed as part of a timebase method, which allows the autosampler to trigger external devices at pre-defined times during a run. Table 6 and Table 7 give the configurations for connectors P2 and P3.

Maximum voltage for the TTL outputs is 5.5 volts. An output voltage of greater than 3.5 V corresponds to logical 1. An output voltage of less than 1.0 V corresponds to logical 0. The sink current is ± 20 mA. All markers are active low (logical 0).

Table 6. Connector P2 TTL MARKER OUTPUTS

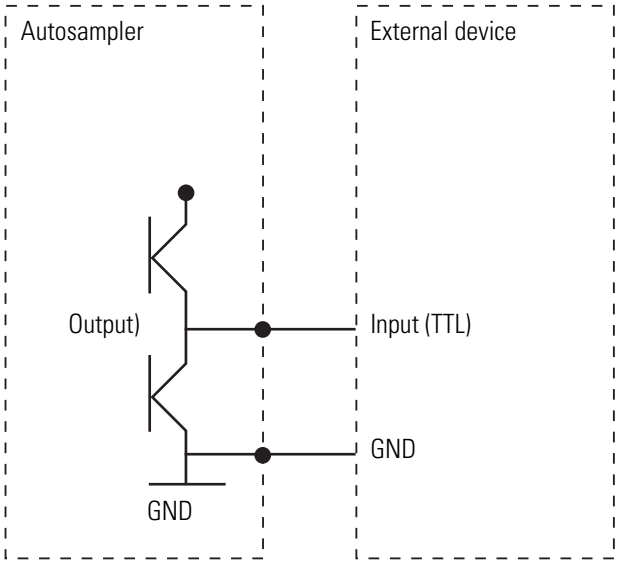
Output	Description
1	INJECT MARKER
2	VIAL/WELL MARKER
3	LABELED WELL MARKER
4	STOP I/O
5	not connected
6	not connected
7	not connected
8	not connected
9	not connected
10	not connected
11	not connected
12	not connected
13	signal ground
14	signal ground
15	signal ground

Note In most modes of operation, a marker output pulse is generated when the injection valve switches from LOAD to INJECT. However, in a user program, markers must be programmed by the user.

Table 7. Connector P3 TIMEBASE OUTPUTS

Output	Description
1	TB 0 (HEX) (1)
2	TB 1 (HEX) (2)
3	TB 2 (HEX) (4)
4	TB 3 (HEX) (8)
5	not used
6	signal ground
7	signal ground
8	signal ground
9	signal ground

Figure 28. TTL output



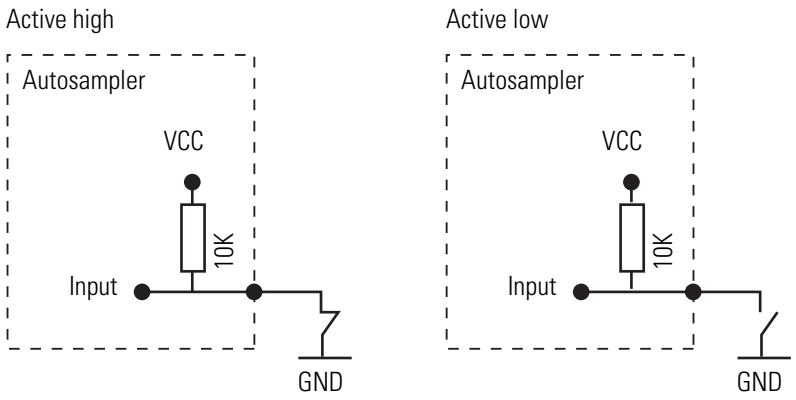
TTL Inputs

Connector P6 is an active high or active low TTL input. Its mode of operation can be specified in the System Menu. The NEXT INJECTION INPUT and the NEXT WELL INPUT can be used when the Micro AS works in REMOTE CONTROL mode. The FREEZE INPUT and STOP I/O input can be used by other devices to control the Micro AS. The four inputs (INPUT 1 to 4) can be used only in the user program, for example to control the sequence of the steps in this method. A connection diagram is shown in Figure 29.

Table 8. Connector P6 TTL INPUTS

Output	Description
1	NEXT INJECTION INPUT
2	NEXT WELL INPUT
3	FREEZE INPUT
4	STOP I/O
5	INPUT 1
6	INPUT 2
7	INPUT 3
8	INPUT 4
9	signal ground
10	signal ground
11	signal ground
12	signal ground
13	signal ground
14	signal ground
15	signal ground

Figure 29. TTL Input



The use of the following TTL inputs is discussed further in this section:

Next Injection Input

When the Micro AS is started in remote control mode, the NEXT INJECTION input starts the next injection sequence. After the injection sequence is finished, the Micro AS waits for the next input. When the autosampler is not in remote control mode and the Ready Menu is active, a NEXT INJECTION input starts the last programmed series. In this case, the Micro AS executes the complete run as if it had been started with the **Start/Stop** key, and will not wait for another NEXT INJECTION input before beginning subsequent injections.

Next Well Input

A NEXT WELL input causes the Micro AS to perform the next injection from the next well, even if not all injections from the current well in the programmed injection method have been executed.

Freeze Input

The FREEZE input causes the Micro AS to freeze the analysis time for the time this input is active. If the FREEZE input is activated while the analysis time is not running, the Micro AS performs all programmed pre-injection sample handling (the mix method and the loading portion of the injection method), but waits to inject the sample until the FREEZE input is no longer active.

Stop I/O

When the STOP I/O input is received, the current run is immediately aborted. If the autosampler is not being operated in remote control mode, it returns to the Ready Menu. If the autosampler is in remote control mode, the run is aborted, and the autosampler remains in remote control mode. In this case, the autosampler cannot be restarted with a NEXT INJECTION input.

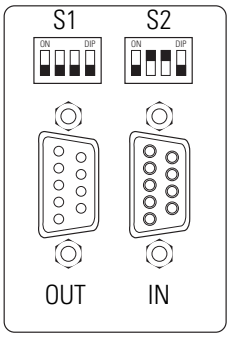
INPUTS 1-4

Inputs 1-4 are programmable inputs that can be used in the user program.

Communications Connectors

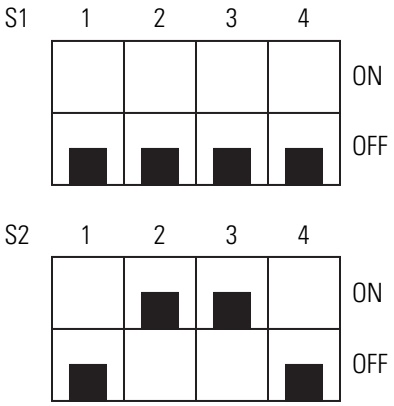
Two communications connectors, labelled S1 and S2, are located at the upper right of the autosampler back panel (See Figure 30.) The communication connectors are standard RS232 or RS422/485 communication interface connectors. Connector S1 is not used with the Xcalibur data system. Connector S2 is used to connect the autosampler to the data system computer.

Figure 30. Communications connectors



Set the dipswitches S1 and S2 as illustrated in Figure 31.

Figure 31. Communications dipswitches, showing correct configuration



The connections for the 9-pin connector are as follows:

- Pin 2 (TD): Transmitted data to the computer.
- Pin 3 (RD): Received data from the computer.
- Pin 5 (SG): Signal ground (also indicated as GND in some devices).

Getting Connected

This chapter describes how to connect the Micro AS to the data system computer and how to interconnect the devices of your Thermo Scientific LC/MS system.

To create an LC/MS instrument, you can use the Micro AS autosampler in combination with the one or two Surveyor MS Pumps and an LTQ Series MS detector. For more information about the Surveyor MS Pump Plus, refer to the *Surveyor MS Pump Plus Hardware Manual*. For more information on connecting an LTQ Series MS detector to LC devices, refer to its Getting Connected manual.

Contents

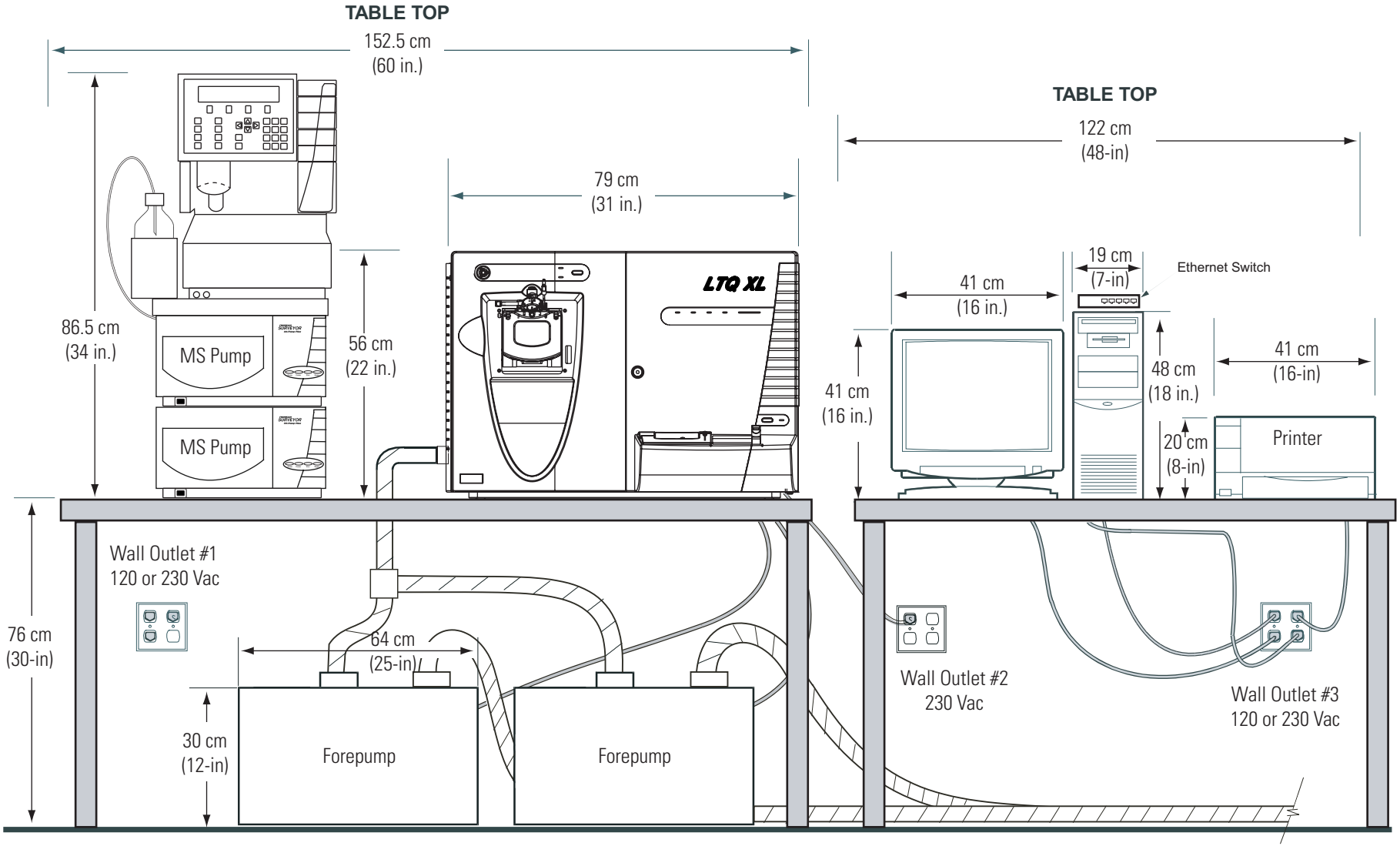
Setting Up the LC/MS System

As Figure 32 shows, an LC/MS system that contains a Thermo Scientific Micro AS autosampler typically contains one or two Surveyor MS Pumps, an LTQ MS detector with vacuum pumps, and a data system computer.

❖ To set up the LC/MS system

1. Place the LC system 3 inches to the left of the LTQ Series MS detector.
2. As shown in Figure 32, stack the LC components as follows:
   a. If your LC system contains a Surveyor MS Pump Plus that will be used as a sample pump, place it on the bottom of the stack.
   b. Place the Surveyor MS Pump Plus that will be used as the HPLC pump on top of the sample pump.
   c. Place the solvent tray on top of the Surveyor MS Pump Plus.
   d. Place the Micro AS autosampler on the top of the stack.

Figure 32. Benchtop view of an LC/MS system with a Micro AS autosampler, two Surveyor MS Pumps, an LTQ Series MS detector, and a data system computer



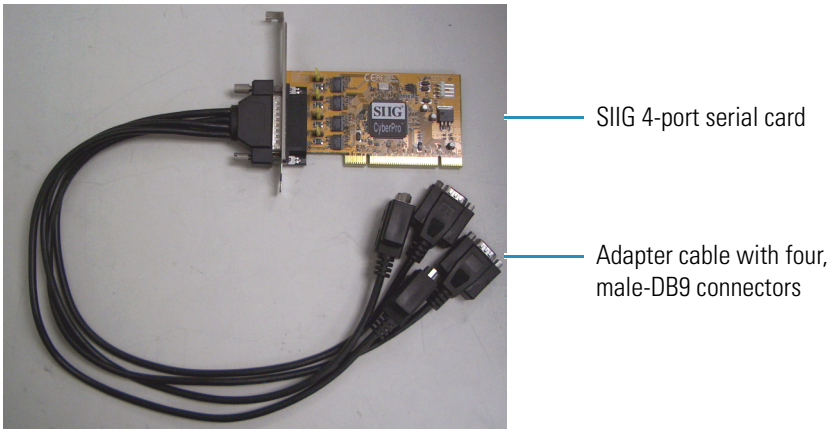
Connecting the LC Devices to the Computer

The Micro AS autosampler and the Surveyor MS Pump Plus communicate with the data system computer through serial communication cables.

The serial communication cable for the Surveyor MS Pump Plus, which is supplied in the accessory kit for the pump, is a standard RS232 cable, approximately 2 m long and terminated at each end with a DB-9 pin connector.

The serial communication ports of the Dell computer cannot handle the data rate required for optimal performance by the Micro AS autosampler and the Surveyor MS Pump Plus. The accessory kit for the Micro AS autosampler contains a hi-speed, 4-port, serial card (shown in Figure 33) with adapter cable that will be installed by a Thermo Fisher Scientific service engineer during the initial installation of your LC/MS system or during an upgrade of your system.

Figure 33. SIIG 4-port serial card and adapter cable



For instructions on how to connect your LTQ Series MS detector to your data system computer, refer to the Getting Connected manual for your LTQ Series MS detector.

❖ To connect the LC devices to the data system computer

1. Ensure that the hi-speed, 4-port, serial card has been installed by a Thermo Fisher Scientific service engineer. See Figure 34.
2. To connect the Surveyor MS Pump Plus (used as the HPLC pump) to the computer:
   a. Connect one end of the serial communication cable to the 9-pin receptacle on the rear panel of the Surveyor MS Pump Plus.
   b. Connect the other end of the serial communication cable to COM port 3 of the adapter cable connected to the computer.
3. To connect the Surveyor MS Pump Plus (used as a sample pump) to the computer:
   a. Connect one end of the serial communication cable to the 9-pin receptacle on the rear panel of the Surveyor MS Pump.

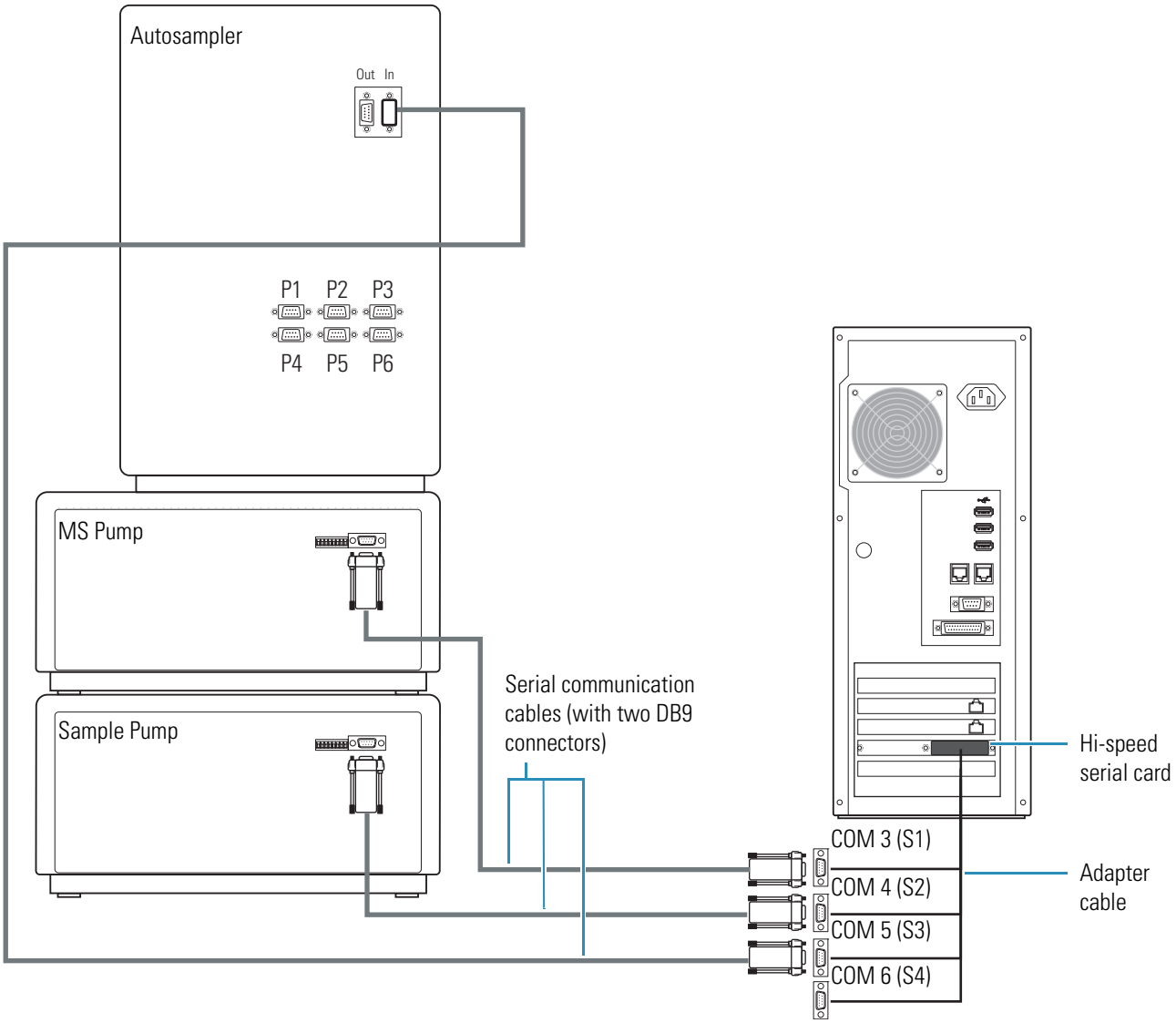
b. Connect the other end of the serial communication cable to COM port 4 of the adapter cable connected to the computer.

4. To connect the Micro AS autosampler to the computer:

   a. Connect one end of the serial communication cable to the 9-pin, IN receptacle on the rear panel of the autosampler.

   b. Connect the other end of the serial communication cable to COM port 5 of the adapter cable connected to the computer.

Figure 34. Serial communication connections

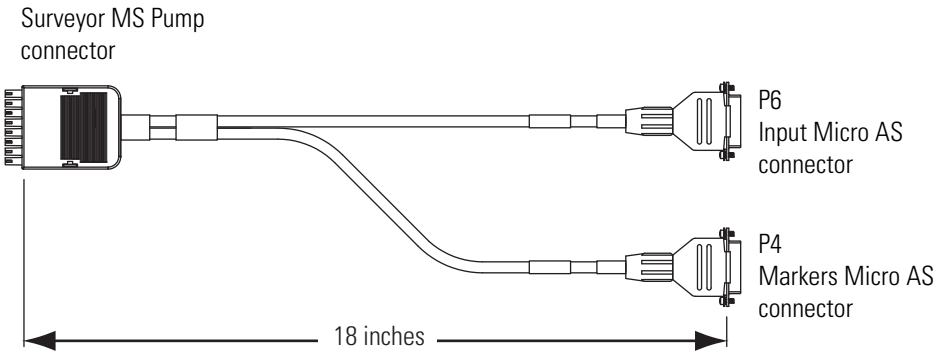


Connecting the Synchronization Cables

The LC/MS system typically consists of the following components: a Micro AS autosampler, one or two Surveyor MS Pumps, and an LTQ series or LXQ mass spectrometer. Two cables, which are included in the accessory kit, are used to synchronize the run signals between the components of the system.

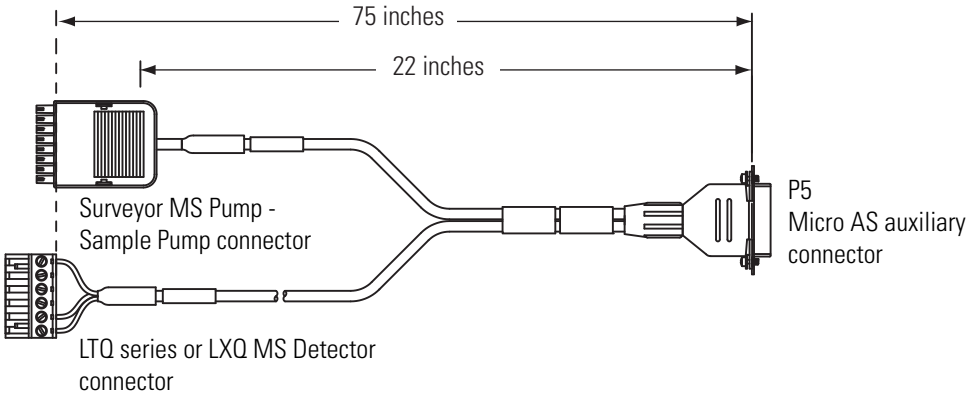
The MicroAS/Pump synchronization cable (P/N 97255-63004), shown in Figure 35, synchronizes the run signals between the Micro AS autosampler and the Surveyor MS Pump.

Figure 35. Synchronization cable for the Micro AS and the Surveyor MS Pump



The Micro AS/MS Detector/Sample Pump synchronization cable (P/N 97255-63000), shown in Figure 36, synchronizes the run signals between the Micro AS autosampler, the MS detector, and an optional Surveyor MS Pump Plus used as a sample pump.

Figure 36. Synchronization cable for the Micro AS, LTQ or LXQ MS detector, and optional Surveyor MS Pump Plus used as a sample pump



❖ To connect the synchronization cables

1. To connect the MicroAS/Pump synchronization cable (P/N 97255-63004) to the Micro AS and the Surveyor MS Pump Plus used as an analytical pump:

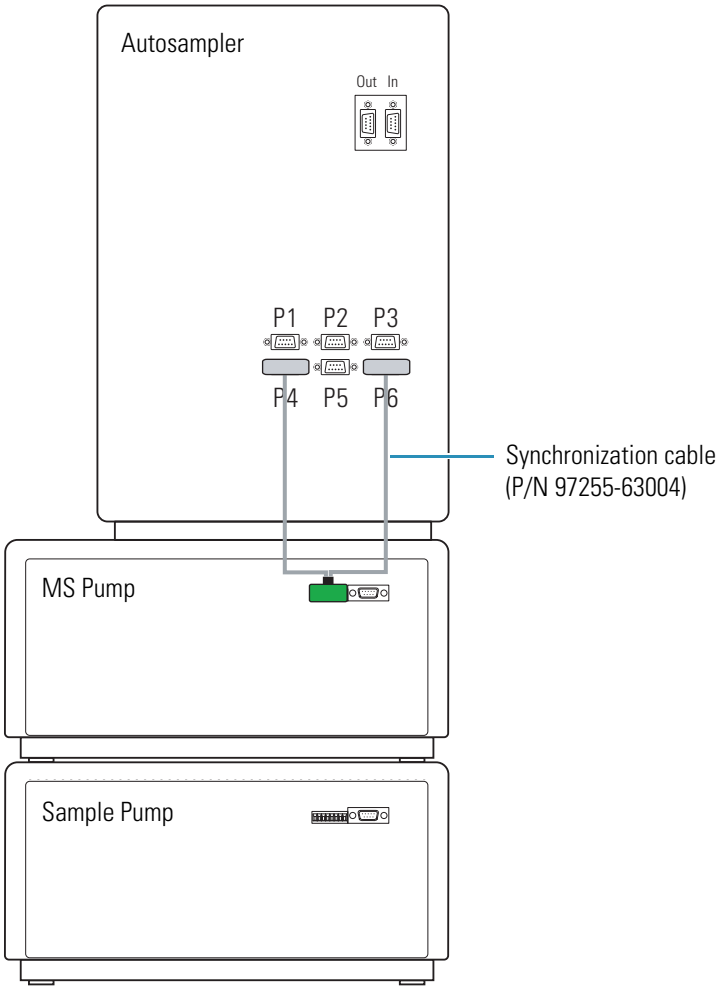
   a. Plug the P4 Markers Micro AS connector into the P4 port on the back panel of the Micro AS autosampler.

   b. Plug the P6 Inputs Micro AS connector into the P6 port on the back panel of the Micro AS autosampler.

   c. Plug the MS pump connector into the 8-pin port on the back panel of the Surveyor MS Pump Plus.

   Figure Figure 37 shows the connections for the MicroAS/MS Pump synchronization cable.

Figure 37. Connections for the MicroAS/MS Pump synchronization cable



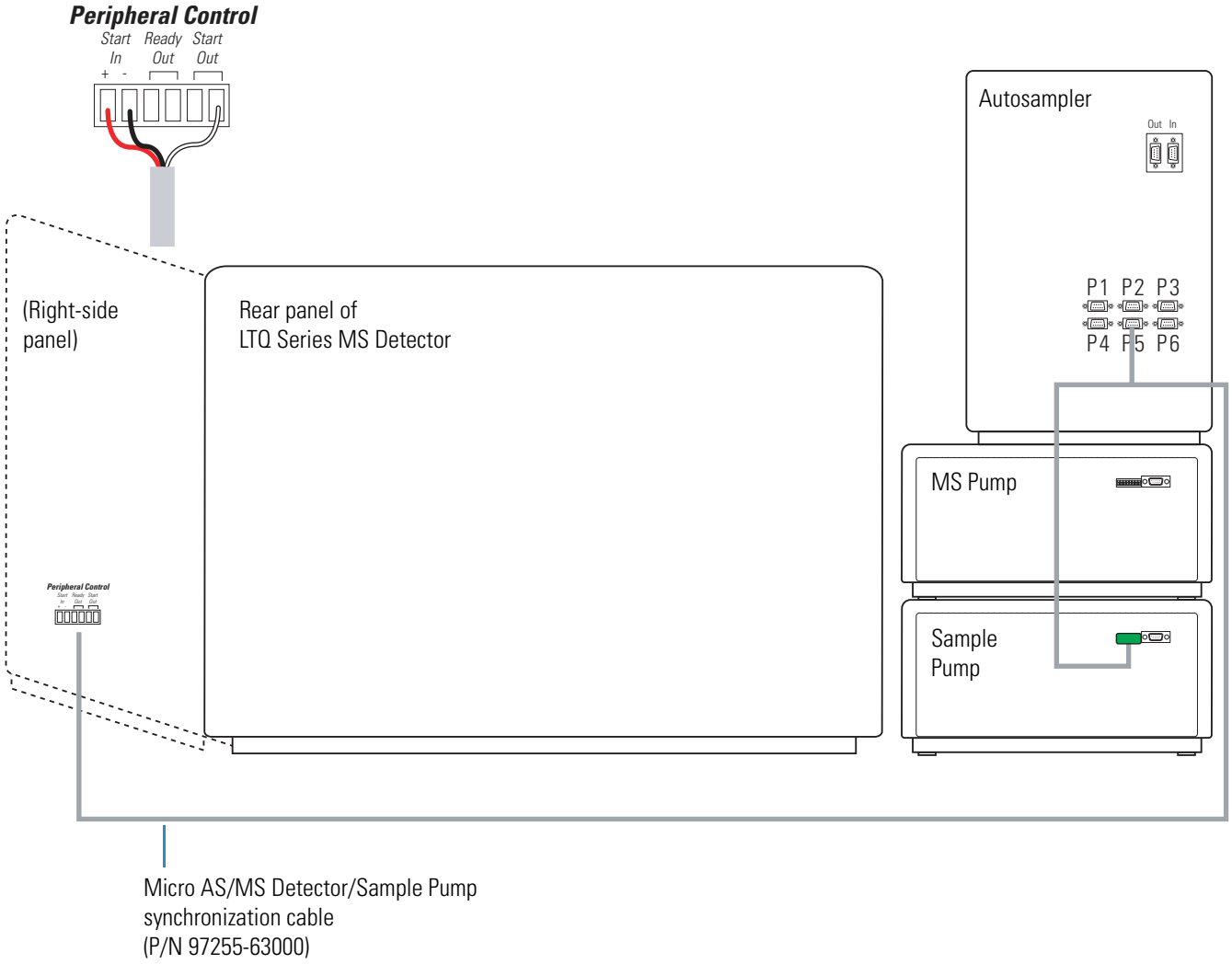
2. To connect the Micro AS/MS Detector/Sample Pump synchronization cable (P/N 97255-63000) to the Micro AS, MS detector, and optional sample pump as shown in Figure 38:

   a. Connect the serial connector to the P5 auxiliary port on the rear panel of the Micro AS autosampler.

   b. Connect the 6-pin connector to the Start In pins on the right-side panel of the LTQ Series MS detector. The red wire connects to the Start In + pin, the black wire connects to the Start In – pin, and the white connects to the Start Out pin as shown in Figure 38.

   c. If the system contains a sample pump, connect the green, combicon connector to the 8-pin port on the rear panel of the Surveyor MS Pump Plus (used as a sample pump). If the system does not contain a sample pump, this connector is left unused.

Figure 38. Connections for the Micro AS/MS Detector/Sample Pump synchronization cable



Preparing for Use

A number of items required for use of the Micro AS are factory-installed (refer to Chapter 2, "Installation," for more information). This chapter describes procedures for the installation of additional parts necessary for autosampler operation.

Contents

Note Ensure that the power is switched on before performing any of the procedures described in this chapter.

HPLC Connections

Make the following connections:

- HPLC pump to port 1 of the injection valve
- HPLC column to port 6 of the injection valve

The instrument was flushed with isopropanol before it was shipped from the factory. Make sure that the mobile phase of your HPLC system is miscible with isopropanol, or start up with an intermediate solvent as mobile phase (disconnect the HPLC column).

CAUTION To ensure optimum injection performance, do not exchange column and pump connections at the injection valve.

Connecting the Waste Tubing

❖ **To connect the waste drainage tubing**

1. Connect the syringe waste tubing by putting the end of the syringe waste tube (see Figure 1 on page 2) into a bottle placed next to the Micro AS.
2. Connect the wash-position drainage tubing:
   a. Connect a length of the silicone tubing (7 mm ID, 10 mm OD) from the parts and accessories kit (P/N 00960-01-00010) to the drain wash connector of the Micro AS (see Figure 1, number 10).
   b. Place the other end of the tubing in a waste container on the floor. All liquid dispensed to waste at the back of the plate is drained through this tubing.
3. Connect the condensed water and solvent drainage tubing:
   a. Connect another length of silicone tubing to the drain port of the Micro AS (see Figure 1, number 11).
   b. Place the other end of the tubing in a waste container on the floor. All leaked solvents and condensed water are drained through this hose.

When operating the autosampler, make sure that the flow path of the waste tubing is not obstructed in any way.

Installing the Wash Solvent Bottle

The Micro AS has a 250 mL wash solvent bottle.

❖ **To install the 250 mL wash solvent bottle**

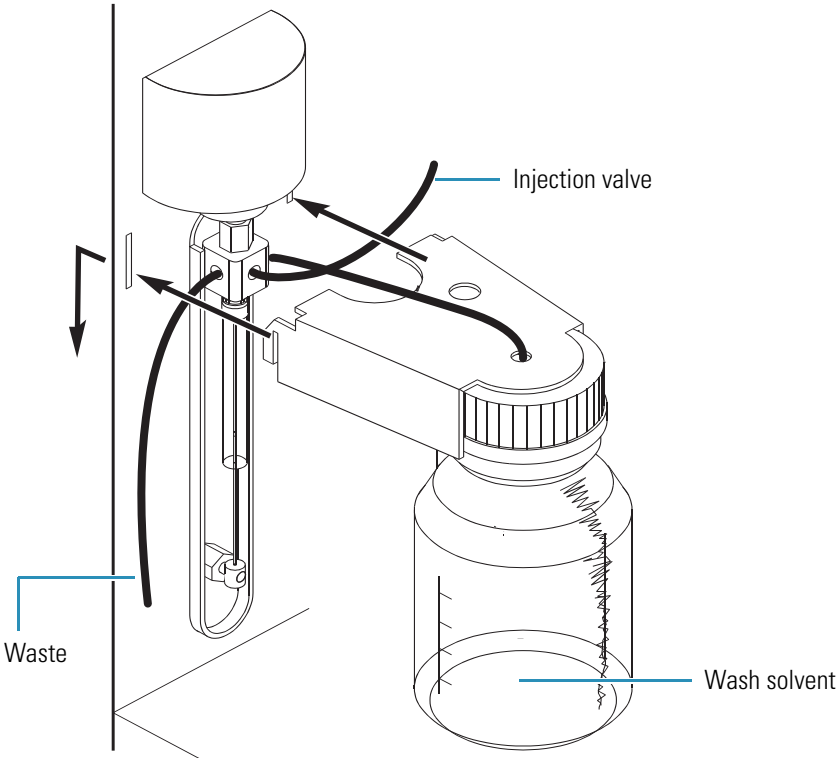
1. Fill the wash solvent bottle with the appropriate wash solvent. Use either methanol (organic solvent, no buffers) or a mixture of water and isopropanol (80/20). Before using the wash solvent, degas it with helium or an ultrasonic bath.
2. Screw the bottle to the cap in the holder.
3. Place the holder in the Micro AS as indicated in Figure 39.
4. Put the wash solvent tube in the wash solvent.
5. Lower the cover of the Micro AS.

6. On the front keypad of the autosampler, press EXIT to exit serial mode and enter the Ready Menu.
7. In the Ready Menu, press the soft function key SYR END to fill the syringe.
8. Press SYR HOME to return the syringe to the home position.
9. Repeat step 7 and step 8 until the wash solvent tube and the syringe are completely filled.
10. Press the soft function key WASH to perform a standard wash routine.
11. If any air remains in the syringe, press SYR END again to fill the syringe with wash solvent. Then press SYR HOME again to move contents to waste. Repeat if there is still air in the syringe and gently tap the syringe as wash solvent is dispensed to waste. (If there is air in the syringe that cannot be dislodged by this method, refer to "Removing an Air Bubble From the Syringe" on page 95.)
12. Press **Menu**, then press SERIAL to place the autosampler back into the serial mode.

Note The autosampler must be placed into serial mode before you attempt to operate the autosampler using the data system control software. The data system computer will not be able to establish communication with the autosampler unless the autosampler is in serial mode.

Place the autosampler in serial mode by pressing the SERIAL soft-function key in the Ready Menu.

Figure 39. Installation of wash solvent bottle



If your experiment requires more than 250 mL of wash solvent for a complete run, install a longer tube (with a flanged end for the valve fitting) and place a larger bottle next to the Micro AS. To fill the larger wash solvent tube, you might have to repeat step 7 and step 8 of the wash solvent bottle installation procedure several times.

Choosing the Sample Loop

When the standard 25 µL syringe is used with the standard 50 µL buffer tubing and the standard 20 µL sample loop, the injection volume ranges shown in Table 9 are available for the various injection modes.

Table 9. Injection volumes with the 20 µL sample loop

Injection method	Injection volume
Full loop	20 µL
Partial loopfill	0.01 to 10 µL
µL pick-up	0.01 to 6.4 µL
µL pick-up (qualitative analysis)	0.01 to 20 µL

An optional 10 µL sample loop is also available for the Micro AS. Using the smaller sample loop can improve the accuracy of partial loopfill injections by minimizing the expansion of loop contents that occurs when the injection valve switches from INJECT to LOAD. Table 10 shows the available injection volumes for the autosampler fitted with the 10 µL sample loop.

Table 10. Injection volumes with the 10 µL sample loop

Injection Method	Injection Volume
Full loop	10 µL
Partial loopfill	0.01 to 5 µL
µL pick-up	0.01 to 1.4 µL
µL pick-up (qualitative analysis)	0.01 to 10 µL

The maximum injection volumes are calculated using the following formulas:

- Full loop:

  injection volume = loop volume

- Partial loopfill:

  max. injection volume = 50% of loop volume

- µL pick-up:

  max. injection volume = (loop volume - 3 × needle volume)/2

Replacing the Syringe

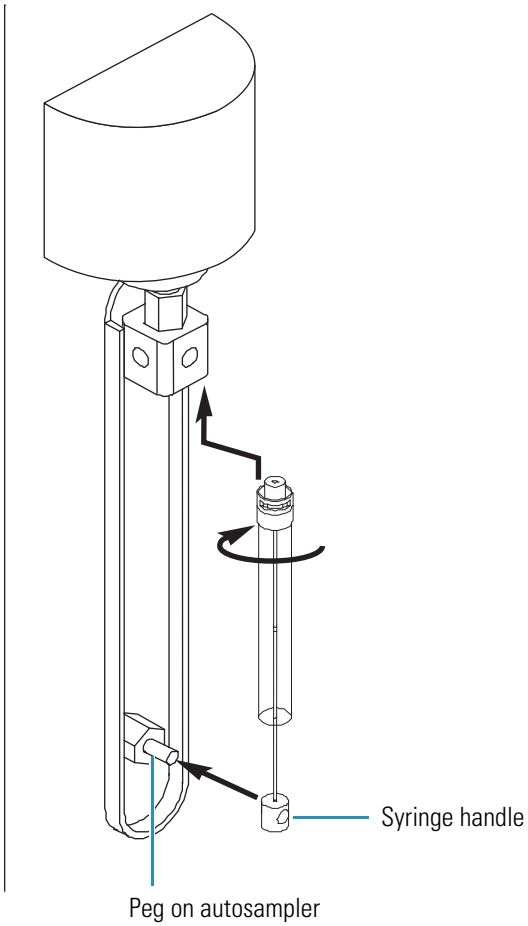
The Micro AS is equipped with a 25 µL syringe.

❖ To replace the syringe

1. On the front keypad of the autosampler, press EXIT to exit serial mode and enter the Ready Menu.
2. In the Ready Menu, press soft function key SYR END to move the syringe to end position.
3. Lift the cover.
4. Unscrew the top of the syringe by turning it clockwise.
5. Pull the bottom of the syringe towards you. You can now remove the syringe (see Figure 40).
6. Fill the new syringe with wash solvent and make sure that all air bubbles are removed from the syringe.
7. Connect the bottom of the filled syringe to the Micro AS autosampler by fitting the hole in the end of the syringe handle over the peg on the autosampler.
8. Screw the top of the filled syringe to the Micro AS by turning counter clockwise.
9. Lower the cover.
10. Press soft function key SYR HOME to remove air from the syringe. The syringe moves to home position and its contents are dispensed to waste.
11. If any air remains in the syringe, press SYR END again to fill the syringe with wash solvent. Then, press SYR HOME again to move content to waste. Repeat if there is still air in the syringe and gently tap the syringe as wash solvent is dispensed to waste. (If there is air in the syringe that cannot be dislodged by this method, refer to "Removing an Air Bubble From the Syringe" on page 95.)
12. In the Ready Menu, press the soft function key WASH to execute a standard wash routine. All tubing connected to the syringe valve will be filled and rinsed.
13. Press **Menu**, and then press the SERIAL soft function key to place the autosampler back into the serial mode.

> **Note** The autosampler must be placed into serial mode before you attempt to operate the autosampler using the data system control software. The data system computer will not be able to establish communication with the autosampler unless the autosampler is in serial mode.
>
> Place the autosampler in serial mode by pressing the SERIAL soft-function key in the Ready Menu.

Figure 40. Installation of the syringe



Replacing the Needle Assembly

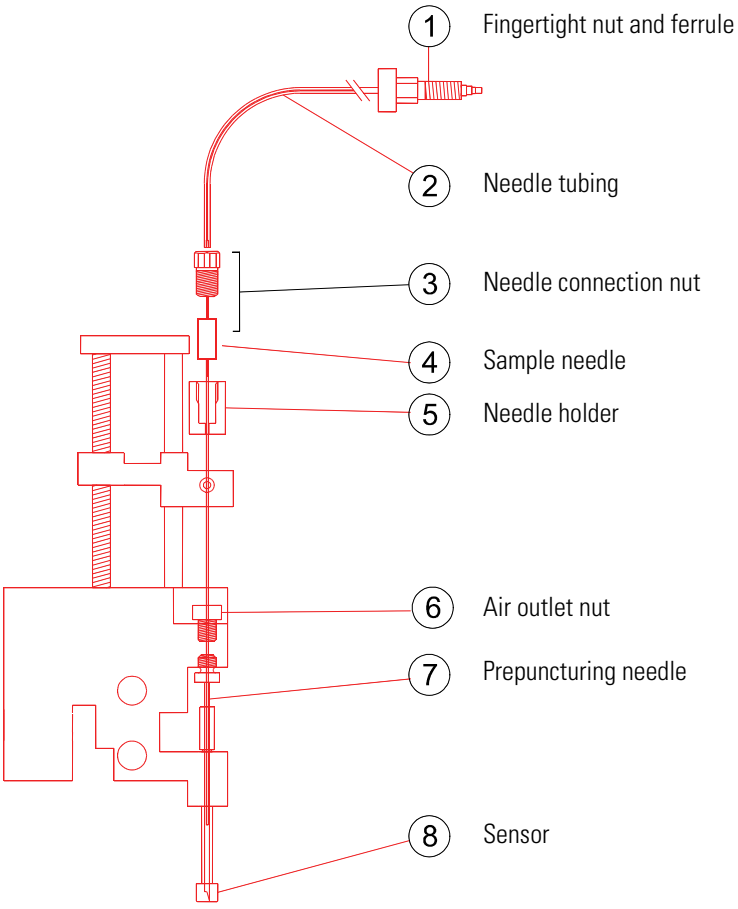
The needle used for sampling consists of two parts:

- A prepuncturing needle, which is a hollow needle used for puncturing of the septum, capmat or sealer. The prepuncturing needle can also used to apply headspace pressure of approximately 0.5 bar (7.25 psi) to the sample.
- A sample needle, which is placed inside the hollow prepuncturing needle and used for the actual transport of sample.

> **Note** Most commercially available sealers or capmats cannot be used in combination with headspace pressure. Switch off headspace pressure (in the General Menu) when using non-compatible sealers or capmats.

Figure 41 illustrates the components of the needle assembly. Parts 1, 2, 3, 4, and 6 constitute the sample needle.

Figure 41. Needle assembly



❖ To replace a needle

1. Loosen the needle connection nut (3).
2. Loosen the fingertight nut and ferrule (1).
3. Carefully pull out sample needle.
4. Insert a new sample needle and tube through the needle holder (5) and tighten the nut (4).
5. Connect the other end of the tube to port 4 of the injection valve using a fingertight nut and ferrule. Do not overtighten the nut, or the tubing may become blocked.
6. Lower the cover of the Micro AS.
7. On the front keypad of the autosampler, press EXIT to exit the serial mode and enter the Ready Menu.
8. Check the sample needle height. (The default height is 5 mm from the plate.) If necessary, adjust the value in the System Menu (General Menu).
9. In the Ready Menu, press the WASH soft function key to clean the new sample needle.

10. Press **Menu**, and then press the SERIAL soft function key to place the autosampler back into the serial mode.

See Figure 41 for the location of the parts in the needle assembly.

Note The autosampler must be placed into serial mode before you attempt to operate the autosampler using the data system control software. The data system computer will not be able to establish communication with the autosampler unless the autosampler is in serial mode.

Place the autosampler in serial mode by pressing the SERIAL soft-function key in the Ready Menu.

Replacing a Sample Plate

The Micro AS allows the use of the following types of plates:

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

A sensor (see Figure 41, number 8) detects the presence of the plate or vials, and provides information on plate height.

Because the Micro AS uses headspace pressure during sample injections, it is very important that samples be properly handled. Note the following:

- Standard wells are best filled using a narrow-end pipette to allow air to escape when filling the well.
- Do not fill wells to the rim. The sample might be forced into the prepuncturing needle and cause cross-contamination of samples and contamination of the sample needle.
- Make sure that the vial seals are airtight to prevent air bubbles in the sample and evaporation of volatile samples. Check the seals after crimping. If the cap can be turned easily, the seal is not airtight, and the handcrimper should be adjusted.

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

❖ To replace a plate in the Micro AS

1. On the front keypad of the autosampler, press EXIT to exit the serial mode and enter the Ready Menu.
2. In the Ready Menu, press PLATES.
3. Press the EXCHANGE soft function key.

   The plate moves to the left.
4. Take out the plate and replace it with another one.
5. Press the PLATE HOME soft function key.

   The plate moves to the operating position again.
6. Do one of the following:
   - If you have replaced the plate with a plate of the same type, go to step 8.
   - If you have installed a new type of plate, go on to step 7.
7. Set the plate parameters:
   a. Press **System**.
   b. Press the PLATES soft function key and press **E**.
   c. Press the soft function key for your plate type.
   d. Press **E**. Press ROWS to process the plate by rows, or press COLUMNS to process the plate by columns.
   e. Press **Escape** to return to the System Menu.
   f. Press GENERAL to enter the General Menu.
   g. Scroll down to the Needle Height parameter, and enter an appropriate needle height for your plate.
   h. Press **Escape** twice to return to the Ready Menu.

      A message appears to indicate that all programmed series will be reset. If you have programmed series using the front keypad, you will need to reprogram them.
8. Press **Menu**, and then press the SERIAL soft function key to place the autosampler back into the serial mode.

Note The autosampler must be placed into serial mode before you attempt to operate the autosampler using the data system control software. The data system computer will not be able to establish communication with the autosampler unless the autosampler is in serial mode.

Place the autosampler in serial mode by pressing the SERIAL soft-function key in the Ready Menu.

Replacing Reagent Vials/Transport Vials

❖ To replace reagent/transport vials

1. On the front keypad of the autosampler, press the EXIT soft function key to exit the serial mode and enter the Ready Menu.
2. Press the soft function key PLATES.
3. Press soft function key EXCHANGE.

   The plate holder moves to the left.
4. Take out the reagent vials/transport vials and replace them with other reagent vials/transport vials.
5. Press soft function key PLATE HOME.

   The plate moves to the operating position again.
6. Press **Escape** to return to the Ready Menu.
7. Press **Menu**, and then press the SERIAL soft function key to place the autosampler back into the serial mode.

Note The autosampler must be placed into the serial mode before you attempt to operate the autosampler using the data system control software. The data system computer will not be able to establish communication with the autosampler unless the autosampler is in the serial mode.

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

In the System Menu, go to the Plates Menu to define the position for first and last Transport vial.

Controlling the Micro AS from Xcalibur

This chapter describes how to use the Xcalibur Instrument Configuration application to configure the Micro AS autosampler, set up an instrument method with the Instrument Setup application, and inject a sample set with the Sequence Setup application.

Table 11 lists the firmware and software requirements for the Micro AS autosampler, the Surveyor MS Pump Plus, the Surveyor MS Pump, and the required version of the Xcalibur data system.

Table 11. Required software and firmware versions

Upgrade	Firmware	Software
Micro AS	1.03	2.00
Surveyor MS Pump	2.47a or higher*	
Surveyor MS Pump Plus	12.47a or higher	1.01.3300 or higher
Xcalibur software	N/A	2.0 SR2 through 2.0.7

*If the firmware version of the pump is less than that listed above, contact your Thermo Fisher Scientific service representative for an upgrade.

To operate the Micro AS from the Xcalibur data system, the autosampler must be in the serial mode. The LCD display on the front panel of the autosampler lists the operation mode as SERIAL as shown in Figure 25 on page 24.

Contents

Configuring the LC/MS System

To control the Micro AS autosampler from Xcalibur, you must add the autosampler to your instrument configuration. This section describes how to create an instrument with a Micro AS autosampler, one or two Surveyor MS Pump Pluses, and an LTQ Series MS detector. In addition, this section describes how to configure the communication, hardware, and tray options for the Micro AS.

Note Connect the Micro AS to the data system computer as described in "Connecting the LC Devices to the Computer" on page 37. When you configure the Micro AS autosampler, take care to select the correct COM port.

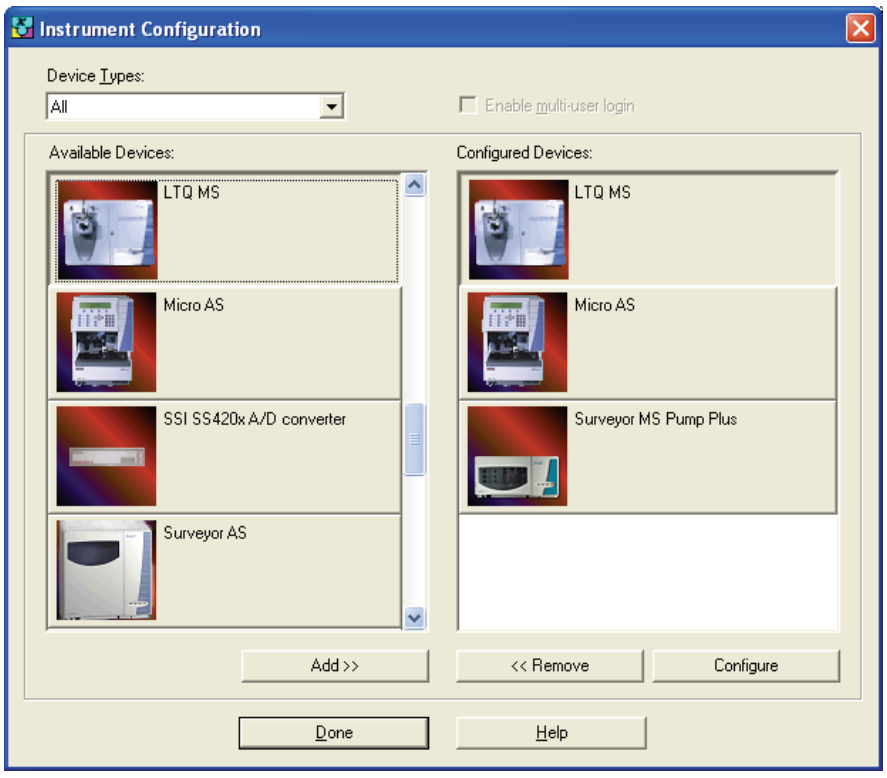
❖ To configure your LC/MS instrument

1. From the Windows XP desktop, double-click the **Instrument Configuration** icon or choose **Start > All Programs > Xcalibur > Instrument Configuration**.

   The Instrument Configuration dialog box appears.

2. Add the devices of your instrument to its configuration as shown in Figure 42:
   a. In the **Available Devices** pane, double-click the **Surveyor MS Pump Plus** button.
   b. In the **Available Devices** pane, double-click the **Micro AS** button.
   c. In the **Available Devices** pane, double-click the **LTQ** button.

Figure 42. Instrument Configuration dialog box, showing the configured devices



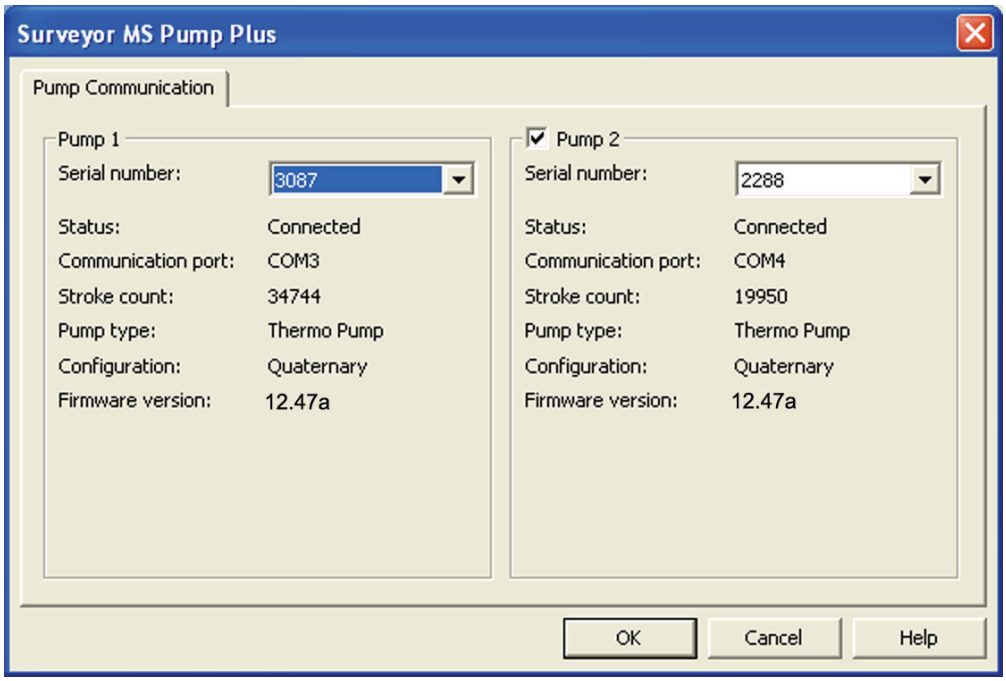
3. In the **Configured Devices** pane, double-click the **Surveyor MS Pump Plus** button.

   The Surveyor MS Pump Plus – Communication page appears. See Figure 43.

4. Confirm the communication port settings for the pump (or dual pump system), and then click **OK** to close the dialog box and return to the Instrument Configuration dialog box.

IMPORTANT The firmware version of the Surveyor MS Pump Plus must be 12.47a or higher. The firmware version of the Surveyor MS Pump must be 2.47a or higher.

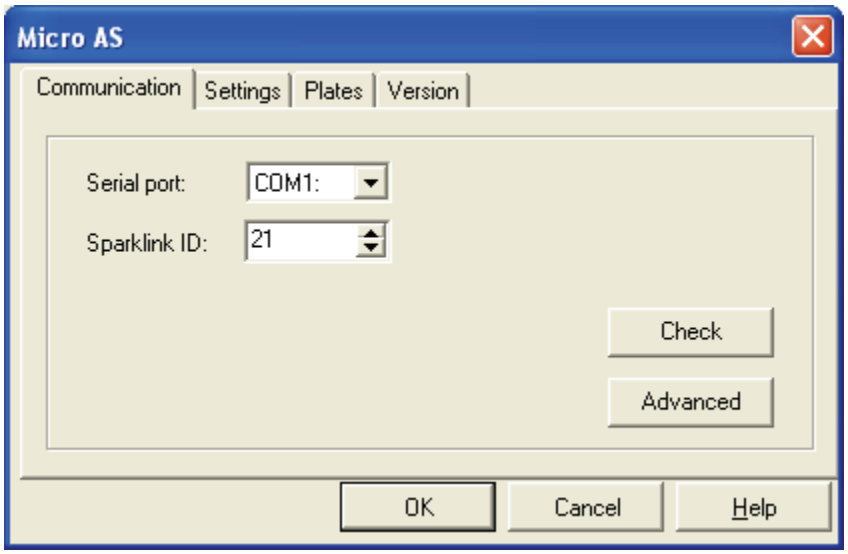
Figure 43. Surveyor MS Pump Plus – Communication page



5. In the **Configured Devices** pane, double-click the **Micro AS** button.

   The Micro AS dialog box appears as shown in Figure 44.

   Figure 44. Micro AS dialog box



6. Select the communication options for the autosampler:
   a. Click the **Communication tab** to open the Communication page.
   b. In the **Serial Port** list, select the serial port used for communication with the Micro AS. See Figure 34 on page 38.
   c. In the **SparkLink ID** box, leave the value at the default of 21.
   d. Click **Check** to ensure that the auto sampler is connected properly and can communicate with the computer.
   e. If the autosampler is communicating with the Xcalibur data system, a dialog box with the message “Micro AS responded” appears.
7. Select the hardware options for the autosampler:
   a. Click the **Settings** tab to open the Settings page.
   b. In the **Autosampler Type** area, select the either Conventional or Micro option.

      Depending on your selection, the available syringe volumes are as follows:

      - For the micro autosampler type, the syringe volume is limited to 25 µL as shown in Figure 45.
      - For the conventional autosampler type, the available syringe sizes are 100, 250, 500, 1000, and 10000 µL. See Figure 46.

   c. In the **Syringe volume (µL)** list, select the syringe volume you want to use for the Conventional autosampler type.

Figure 45. Settings page for the Micro autosampler type

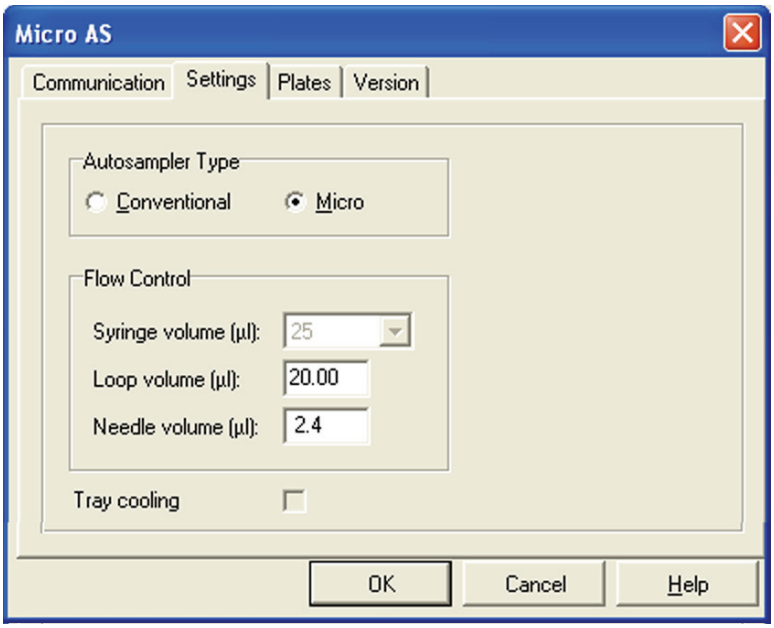
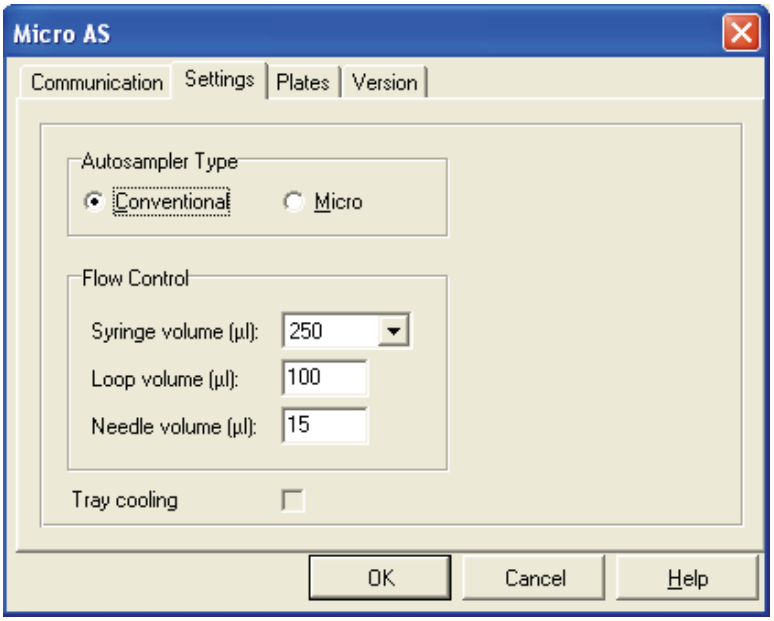


Figure 46. Settings page for the Conventional autosampler type



d. In the **Loop volume (µL)** box, type the nominal sample loop volume.

- For the Micro autosampler type, the allowable loop volumes are 0.01 through 20.00 µL, in increments of 0.01 µL.
- For the Conventional autosampler type, the allowable loop volumes are 5 through 1000 µL, in increments of 0.10 µL.

e. In the **Needle volume** box, type the needle volume.

- For the Micro autosampler type, the allowable needle volumes are 0 through 99.9 µL in 0.1 µL increments, with 2.4 µL as the default.

The Micro AS is supplied with a needle that has an internal volume of 2.4 µL.

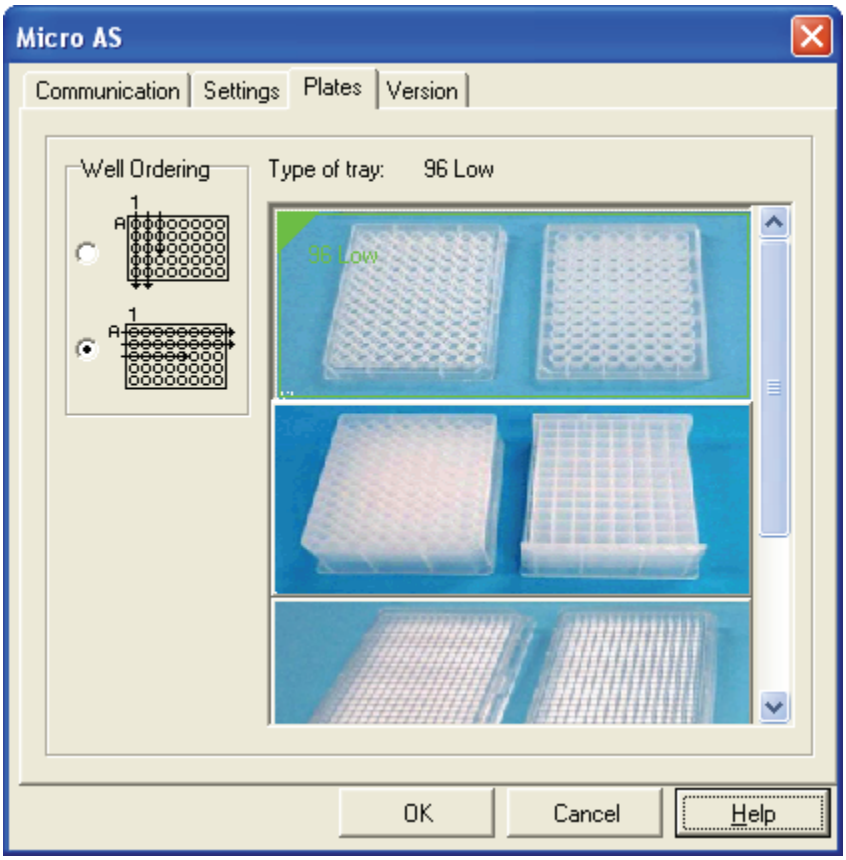
- For the Conventional autosampler type, the allowable needle volumes are 1 through 200 µL in 1 µL increments, with 15 µL as the default.

f. To enable tray cooling, select the Tray cooling check box.

8. Select the plate options for the autosampler:

a. Click the **Plates** tab to open the Plates page shown in Figure 47.

Figure 47. Micro AS – Plates page



b. In the **Well Ordering** area, select the order in which the wells must be processed by selecting either the By columns or the By rows option.

c. In the **Type of tray** list, select the tray type that you are using by clicking its associated picture. The name of the selected tray type is displayed above the list.

9. Click the **Version** tab to open the Version page and check the firmware version of your Micro AS autosampler.

10. Click **OK** to exit the Micro AS dialog box.

11. See the Help provided in the software for information on configuring your LTQ Series MS detector.
12. Click **Done** to exit the Instrument Configuration application.

Creating Instrument Methods

The following procedures describe how to set up an instrument method for an LC system containing a Micro AS, a Surveyor MS Pump Plus, and an LTQ Series MS detector:

- Setting Up the Micro AS Method Parameters
- Synchronizing the Pump with the Micro AS

Note To synchronize the timing of the Micro AS autosampler, the Surveyor MS Pump Plus, and the LTQ Series MS detector during a run, ensure that you specify the following settings in your instrument methods:

- A 3 s duration time (or greater) for the contact closure signal sent by the autosampler as described step 4 on page 64.
- Surveyor AS injection logic for the start setting for the MS pump.

Setting Up the Micro AS Method Parameters

To set up the Micro AS method parameters, perform the following procedures:

1. Specifying the General Parameters
2. Specifying the Injection Parameters
3. Specifying the Wash Parameters
4. Specifying the Timed Events

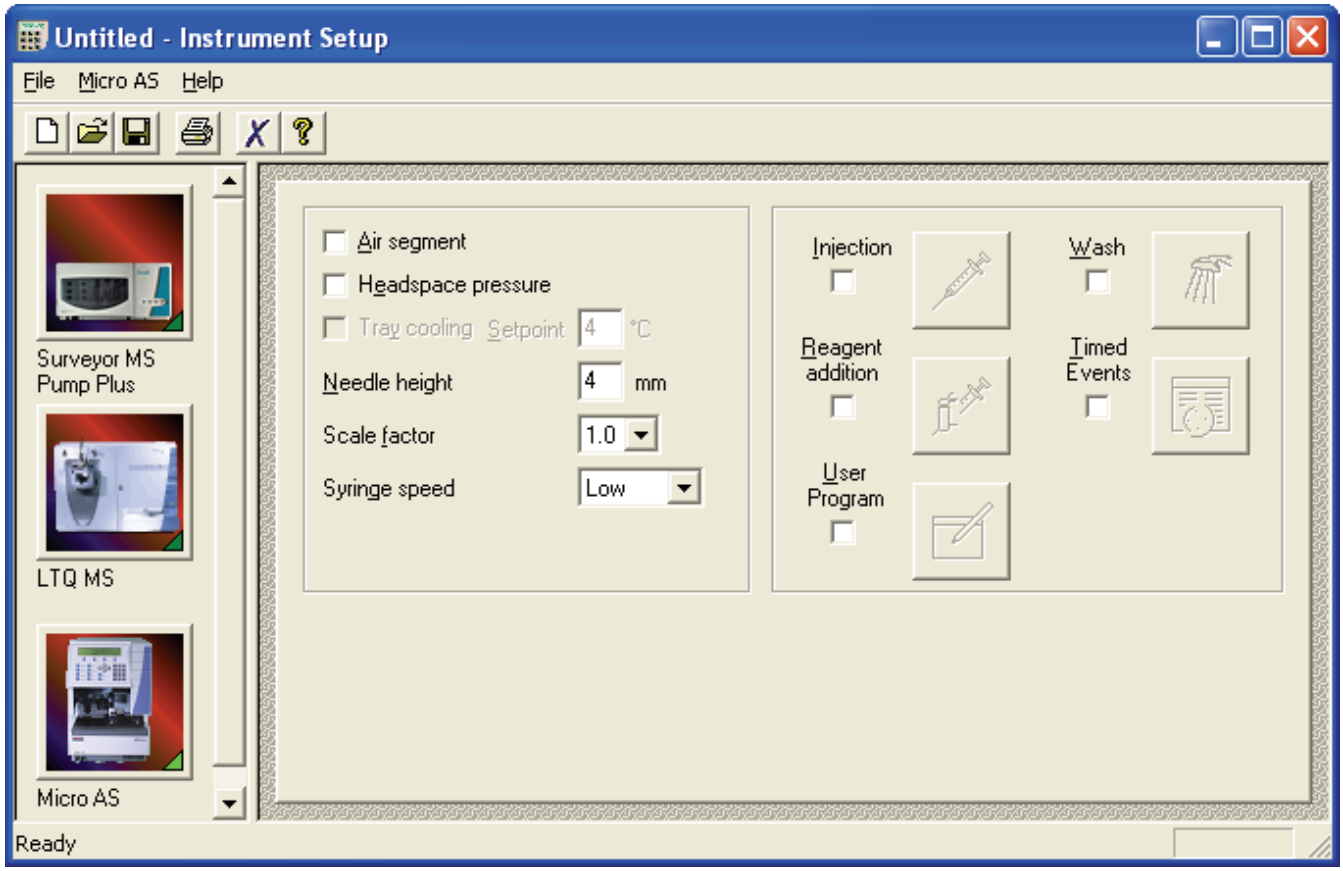
Specifying the General Parameters

❖ To specify the general parameters for the Micro AS

1. In the view bar of the **Instrument Setup** window, click the **Micro AS** button.

   The Micro AS Instrument Setup view appears as shown in Figure 48.

Figure 48. Instrument Setup window – MicroAS view



2. If you are using Partial loopfill or Full loopfill injection method, select the **Air Segment** check box to draw an air segment of 5 µL before the flush volume prior to an injection. The air segment is pushed out of the sample loop before the injection valve switches to the inject position.

   Thermo Scientific recommends that you deselect the Air Segment option for µL Pickup injection methods. For µL Pickup injection methods, the sample will be preceded by an air segment which will be injected into the analytical system.

3. If the sample wells for your trays are airtight (for example, vials with caps), select the **Headspace pressure** check box to increase the accuracy and reproducibility of your injections.

4. To enable tray cooling, select the **Tray cooling** check box, and then type the cooling temperature in the **Setpoint** box.

5. In the **Needle height** box, type a value in mm for the distance between the needle tip and the plate holder.

   Allowable values are 0 to 40 mm. The lower the value, the further the needle tip descends. You risk breaking the needle by entering too low a value. See "Checking the Needle Position" on page 70 for instructions on how to determine an appropriate needle height for your application.

6. In the **Scale factor** list, select a scaling factor that you want to reduce the syringe speed by.

7. In the **Syringe speed** list, select a syringe speed of Low, Normal, or High.

8. For a typical injection, select the **Injection**, **Wash**, and **Timed Events** check boxes.

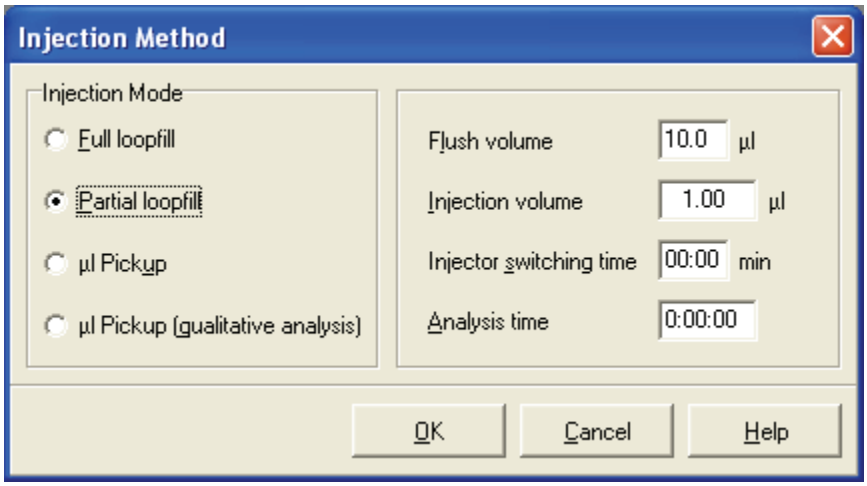
Specifying the Injection Parameters

❖ To specify the injection parameters

1. Click the **Injection** button.

   The Injection Method dialog box appears.

   Figure 49. Injection Method dialog box



2. In the **Injection Mode** area, select the injection mode:

   - In the Full loopfill injection, the autosampler flushes the needle with the sample solution, and then overfills the loop before injecting the contents of the loop into the mobile phase stream. The injection volume is equal to the actual loop volume. The amount of sample withdrawn from the sample well depends on the configured loop size as described in Table 12 and the flush volume specified in the Flush volume text box.

Table 12. Amount of sample withdrawn for Full loopfill injections

Configured loop volume	Amount of sample withdrawn
< 100 µL	3 × nominal loop volume + flush volume
100 µL to 500 µL	2 × nominal loop volume + flush volume
> 500 µL	1.5 × nominal loop volume + flush volume

- In the Partial loopfill injection mode, the autosampler can inject a variable amount of sample up to 50% of the configured loop volume. Before the autosampler meters the requested injection volume into the loop, it flushes the needle with the sample solution. You specify the flush volume in the Flush volume box.
- In the µL Pickup mode, the autosampler sandwiches the sample between two segments of transfer buffer (reagent A vial). The maximum injection volume is described by the following equation:

  $\text{inj volume}_{max} = 0.5 \times \text{configured loop volume} - 1.5 \times \text{needle volume}$

  For a 20 µL loop, the maximum injection volume in the Micro mode is 6.4 µL. The autosampler withdraws the requested injection volume from the well.
- In the µL Pickup (qualitative analysis) the autosampler sandwiches the sample between a segment of transfer buffer (reagent A vial) and the valve seat. Because the headspace option is not used with this injection mode, the injection volume is less precise than that for the µL Pickup mode.

Note For more information on injection methods, refer to "Injection Methods" on page 9.

3. In the **Flush volume** box, type the appropriate flush volume for the Full or Partial loopfill injection mode.

   For optimal results, use a flush volume equal to at least twice the needle volume. The needle used for the Micro mode has a volume of 2.4 µL.

4. In the **Injection volume** box, type the injection volume for the Partial and µL Pickup injection modes.

   For low-flow gradient methods, use the Injector switching time box and the Analysis time box to reduce the gradient delay volume of the system by removing the sample loop from the mobile phase flow path during the gradient portion of the gradient program.

5. In the **Injector switching time** box, do one of the following:

   - To leave the injection valve in the inject position after the autosampler makes an injection, leave the time set to the default of 0:00.

- To switch the injection valve back to the load position after the autosampler makes an injection, type a time in minutes and seconds. To prevent the injection valve from switching back to the inject position before the end of the run, you must also specify an appropriate value in the Analysis time box.

  IMPORTANT Allow for a sufficient length of time for the mobile phase to sweep the sample out of the loop before isolating the loop from the mobile phase flow path. The minimum time that the valve should remain in the inject position depends on the loop volume and the flow rate.

6. In the **Analysis time** box, do one of the following:
   - If the value in the Injector switching time box is set to 0:00:00, leave the analysis time set to the default of 0:00:00.
   - If the method switches the injection valve to the load position (specified in the Injector switching time box), specify an analysis time greater than the injector switching time so that the injection valve remains in the load position. For optimal results, specify an analysis time that leaves the injection valve in the load position until the column regeneration portion of the gradient program or until the end of the run.
7. Click **OK** to finalize the injection settings.

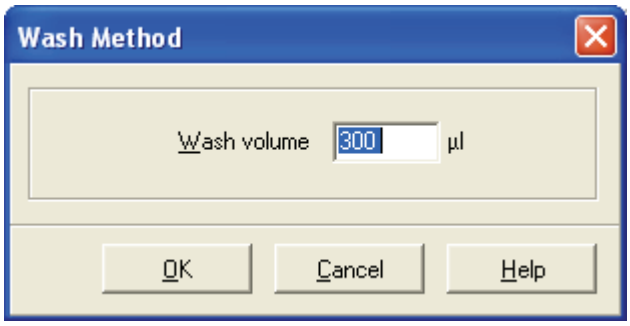
Specifying the Wash Parameters

❖ **To specify the wash parameters**

1. Click the **Wash** button.

   The Wash Method dialog box appears.

   Figure 50. Wash Method dialog box



2. In the **Wash volume** box, type a value for the amount of solvent that will be used to wash the needle after a sample injection.
3. Click **OK** to finalize the wash settings.

Specifying the Timed Events

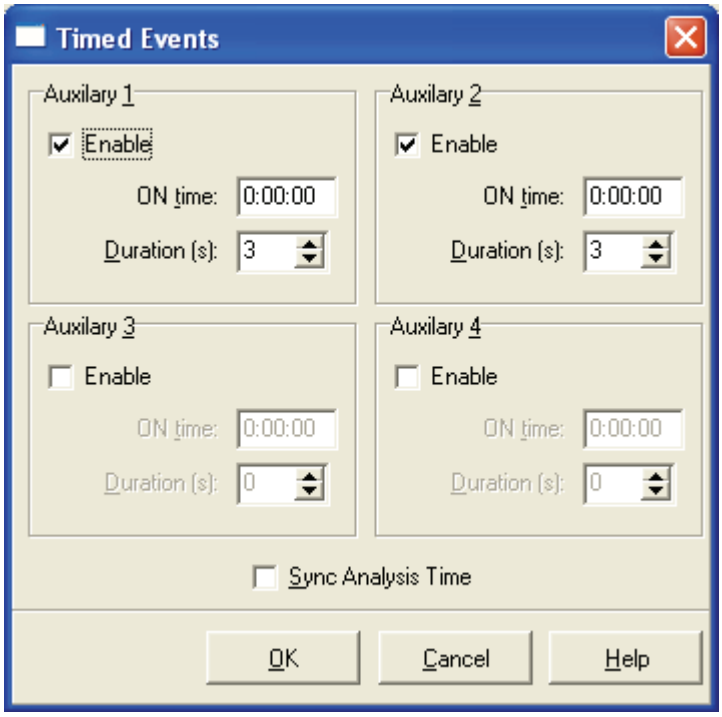
Set up the timed events so that the autosampler and MS detector are synchronized during a run.

❖ To specify the timed events

1. Click the **Timed Events** button.

   The Timed Events dialog box appears. Figure 51 shows the appropriate settings.

   Figure 51. Timed Events dialog box, showing the appropriate settings



2. Select the **Enable** check boxes for auxiliary 1 and auxiliary 2.
3. Leave the ON time at the default of 0:00:00.
4. In the **Duration(s)** box, type **3**.
5. Do **not** select the Sync Analysis Time check box.
6. Click **OK** to finalize the timed event settings.
7. Click **OK** to return to the Micro AS Instrument Setup page.

Creating User Programs

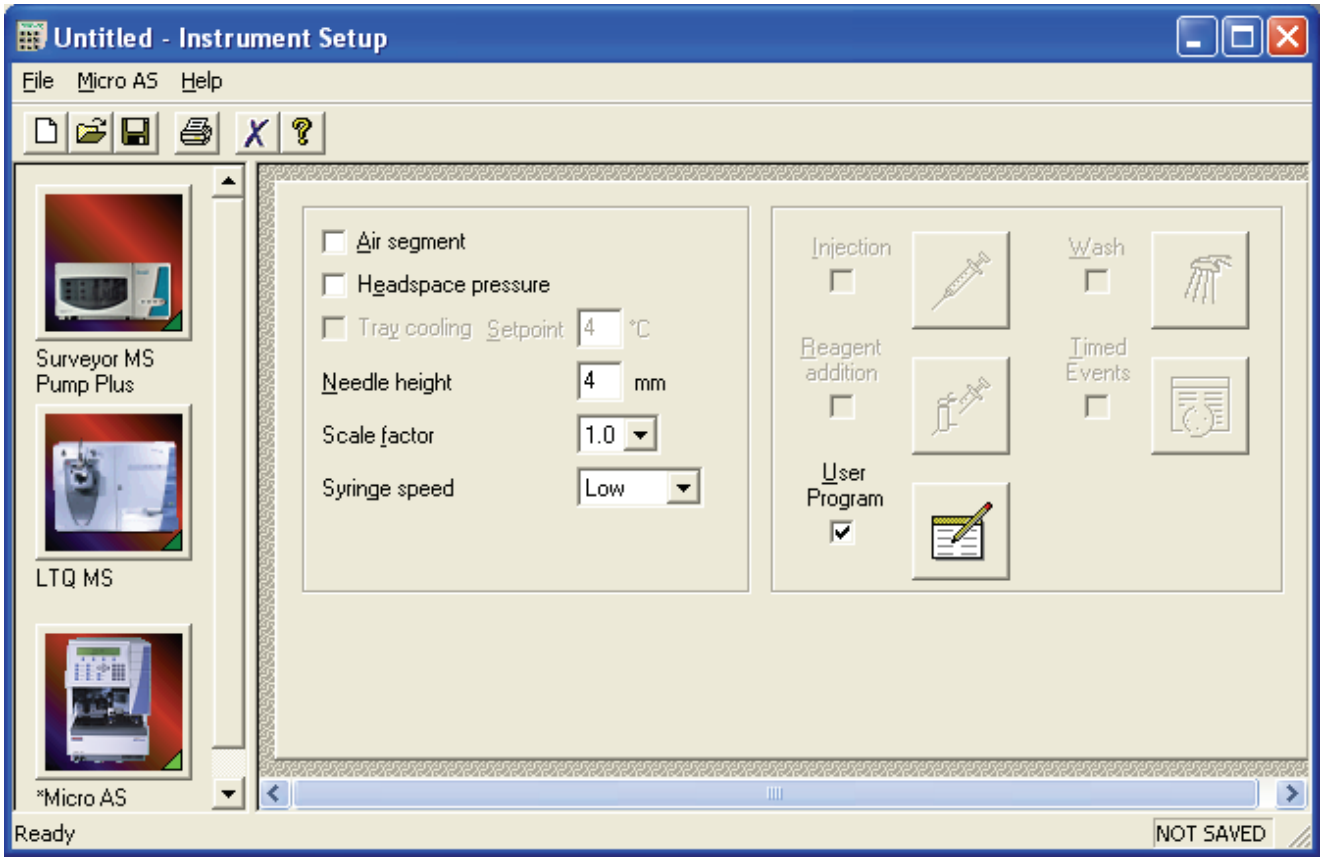
In addition to the standard method parameters for the Micro AS autosampler, you can create user programs to perform specialized functions such as sample preparation.

❖ **To add a user program for the autosampler to the instrument method**

1. Open the Micro AS Instrument Setup view as described on page 59.
2. Select the **User Program** check box as shown in Figure 52.

   Selecting the User Program check box makes the Injection, Wash, Reagent addition, and Timed events check boxes unavailable.

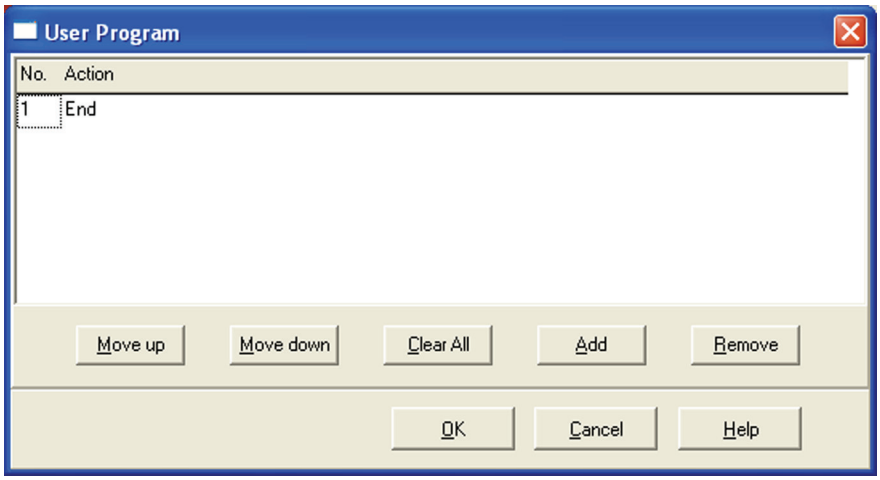
Figure 52. Instrument Setup window – Micro AS view



3. Click the **User Program** button.

   The User Program dialog box appears as shown in Figure 53.

   Figure 53. Default user program

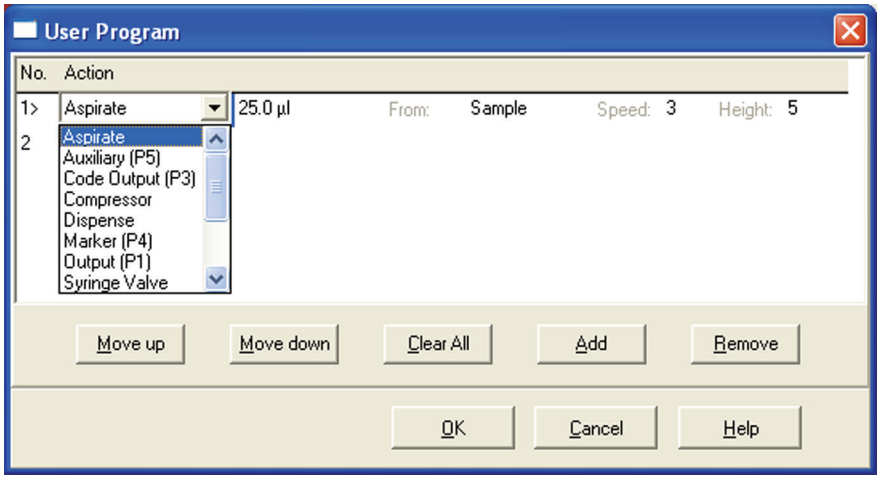


4. To add a step, click the No. column in the row where you want to add a step and click **Add**.

5. To specify an action, click the **Action** column in the row that you want to modify.

   A list of actions appears as shown in Figure 54.

   Figure 54. User Program dialog box, showing the list of actions



6. Select an action and specify its settings, as necessary.

7. To copy a line or a set of lines:

   a. Right-click the line that you want to copy and choose copy line(s).

   b. Right-click the line where you want to add a step and choose paste line(s).

8. Use the Move up, Move down, Clear All, and Remove buttons to edit the spreadsheet.
9. Click **OK** to exit the User Program dialog box.
10. Save the method.

To use an instrument method containing a user program with aspirate or dispense actions, you must modify the sequence spreadsheet as described in "Modifying the User Labels for a User Program" on page 71.

Note Always add a wait step of at least 5 seconds following an aspirate or dispense step that is being used to quantitatively transfer fluid.

For more information on user programs, refer to Appendix F, "1User Programs."

Synchronizing the Pump with the Micro AS

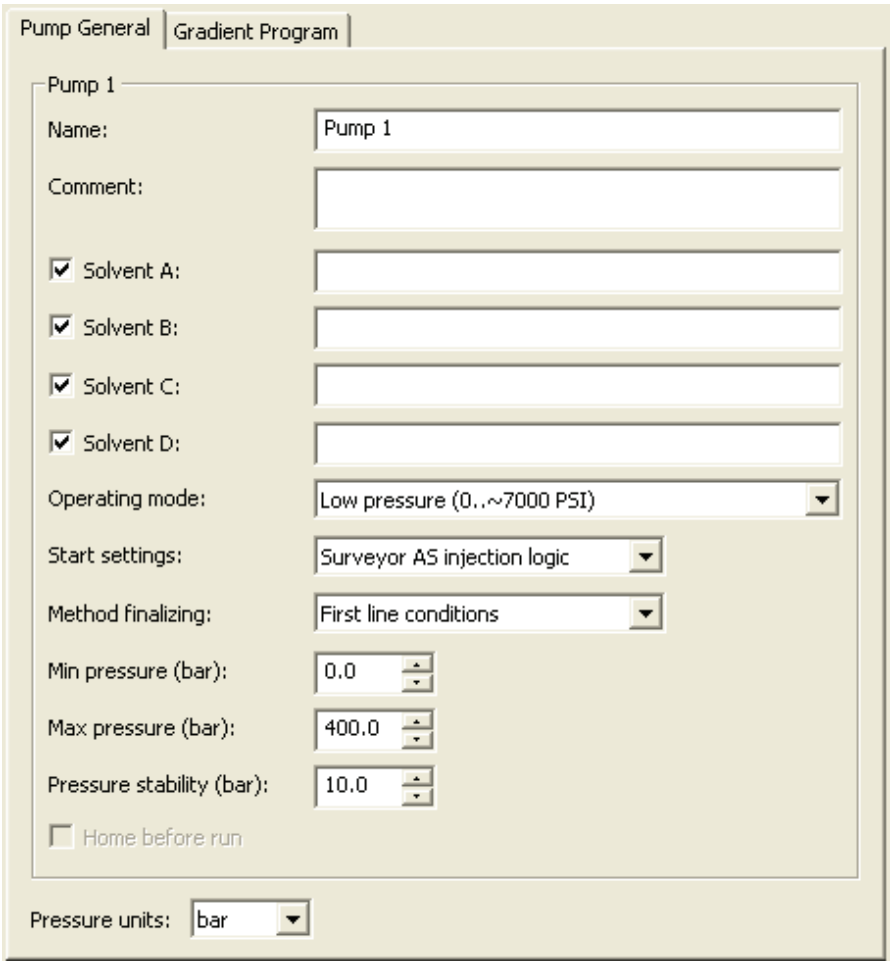
This topic describes how to set up the method parameters for the Surveyor MS Pump Plus so that the timing of the pump during a run is synchronized with the Micro AS autosampler.

❖ To synchronize the Surveyor MS Pump Plus with the Micro AS

1. Open the Surveyor MS Pump Plus Instrument Setup view by clicking the Surveyor MS Pump Plus button in the view bar.

   The Instrument Setup view for the Surveyor MS Pump Plus appears as shown in Figure 55.

Figure 55. Instrument Setup – Surveyor MS Pump Plus – Pump General page, showing the default settings



2. In the **Start settings** list, select **Surveyor AS injection logic**.

   The pump cams reach the home position before the autosampler switches the injection valve to the inject position.

   IMPORTANT If you have not upgraded the firmware version for your Surveyor MS Pump to 2.47a or your Surveyor MS Pump plus to 12.47a, this feature will not work properly, and you will notice a significant reduction in retention time reproducibility.

3. In the **Method finalizing** list, select **First line conditions**.
4. Make the appropriate entries and selections for the other parameters in this page.
5. Save the instrument method.

Using the Direct Controls

You can use the controls in the Direct Control dialog box to perform maintenance, prepare the autosampler for a run, make single injections, or determine an appropriate needle height for the vials or microtitre plates you are using.

This section contains the following procedures:

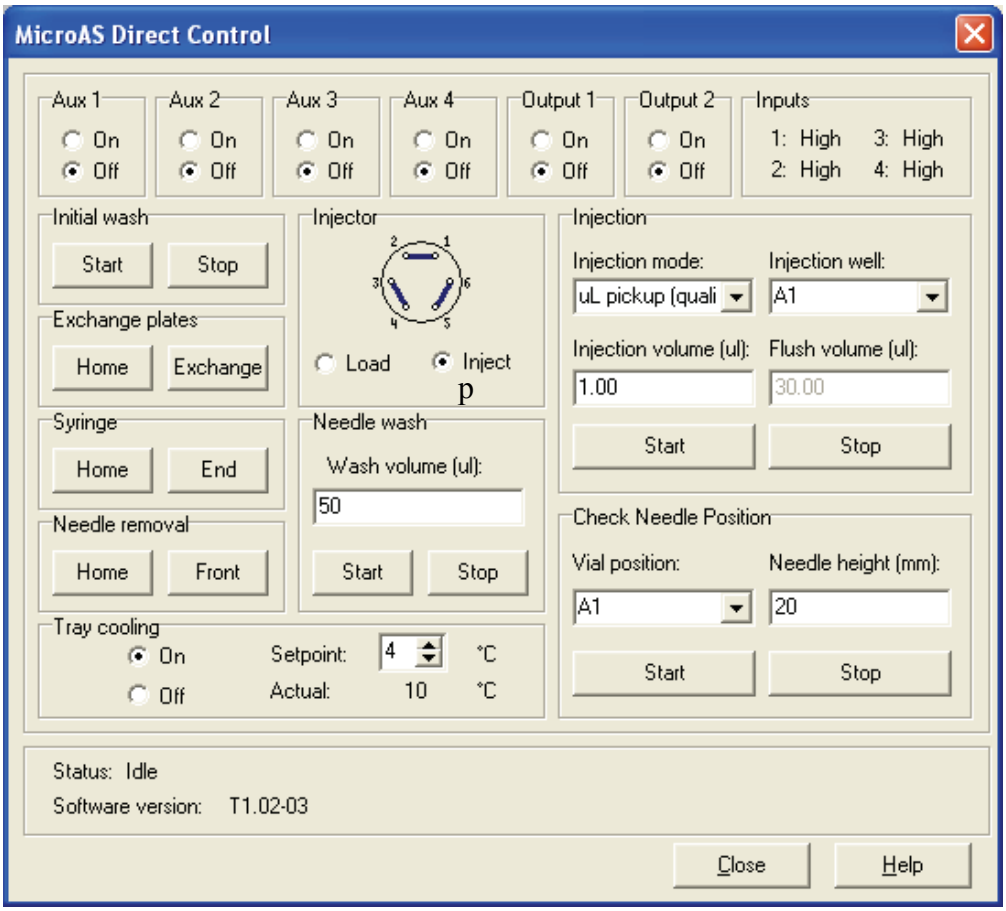
- Opening the MicroAS Direct Control Dialog Box
- Replacing Sample Plates
- Checking the Needle Position

Opening the MicroAS Direct Control Dialog Box

❖ To open the MicroAS Direct Control Dialog Box

From the Micro AS – Instrument Setup view, choose **Micro AS > Direct Control**. The MicroAS Direct Control dialog box shown in Figure 56 appears.

Figure 56. MicroAS Direct Control dialog box



Replacing Sample Plates

❖ **To replace a sample plate**

1. Open the Micro AS Direct Control dialog box.
2. In the **Exchange Plates** area, click **Exchange**.

   The plate holder moves to the left side of the tray compartment.
3. Replace the sample plate.
4. In the **Exchange Plates** area, click **Home**.

The plate holder moves back to the right side of the tray compartment.

Checking the Needle Position

❖ **To determine an appropriate needle height for your application**

1. Open the Micro AS Direct Control dialog box.
2. In the **Check Needle Position** area, do the following:
   a. In the **Vial position** box, type the vial position where you have placed a vial or a well location.
   b. In the **Needle height** box, type the distance from the plate holder that you want the needle tip to descend to. Start with the default value of 20 mm.
   c. Click **Start**.
3. Watch how far the needle tip descends.
4. Repeat steps 1 and 2 using a lower value in the Needle height box until you see a slight deflection of the needle.
5. Record this value. If you have limited sample and you want the needle to descend to the bottom of the vial or well, use this needle height. If you do not want the needle tip to touch the bottom of the vial or well, use a higher value for the needle height.

Using the Sequence Setup Application

This section describes how to run a sample set using a Micro AS autosampler and a Surveyor MS Pump Plus as the inlet LC to an LTQ MS detector.

This section contains the following topics:

- Starting a Sequence Run

Creating a Sequence Spreadsheet

❖ **To create a sequence spreadsheet**

1. From the Roadmap – Homepage view, click the Sequence Setup icon.
2. To create a new sequence:
   a. Choose **File > New**.

      The New Sequence Template dialog box appears.

   b. Make the appropriate selections and entries in the New Sequence Template dialog box, and then click **OK**.

   c. Save the sequence.
3. Verify that the instrument method contains the appropriate parameters:
   a. In the sequence spreadsheet, right-click the instrument method and choose **Open > File**.

      The Instrument Setup window appears.

   b. In the **Pump General** page, ensure that Surveyor AS logic is selected.

   c. Click the **Micro AS** button in the view bar.

   d. If this is not a user defined program, ensure that the contact closure duration setting for the Micro As autosampler is set to 3 seconds or more as shown in Figure 51 on page 64.

   e. Close the Instrument Setup window and return to the Sequence Setup window.

Modifying the User Labels for a User Program

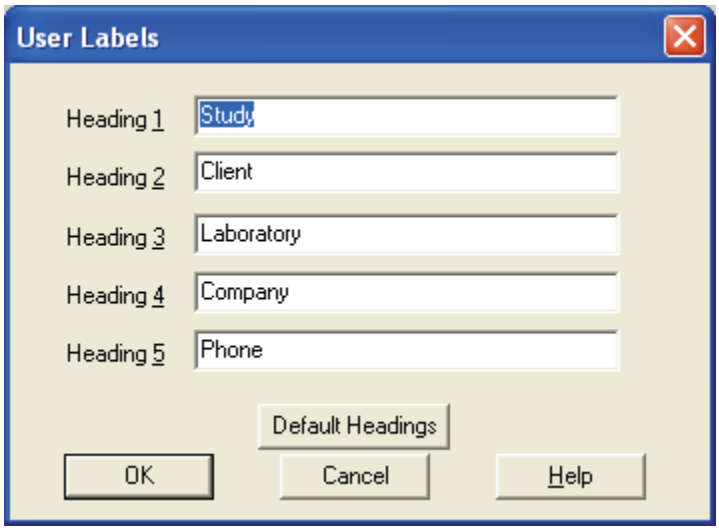
If the autosampler portion of the instrument method contains a user program with an aspirate or dispense step, modify the user labels in the sequence spreadsheet.

❖ **To modify the user labels**

1. In the Sequence Setup window, with the sequence open, click the **User Labels** button.

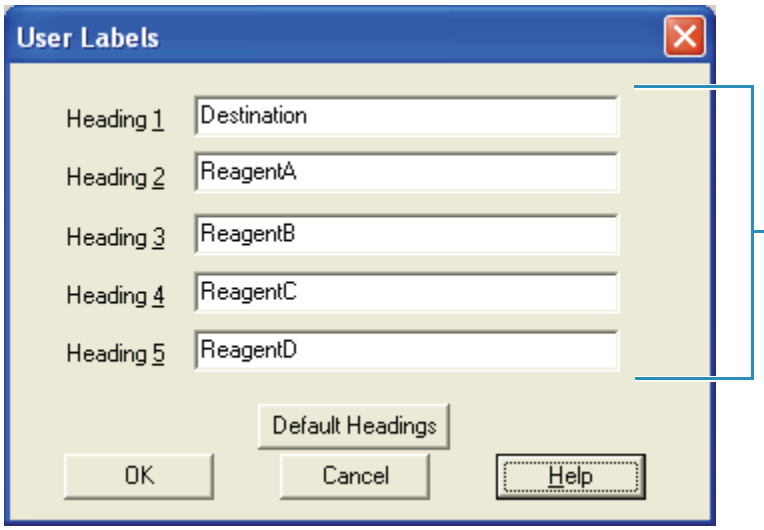
   The User Labels dialog box appears as shown in Figure 57.

Figure 57. User Labels dialog box, showing the default headings



2. Change the user labels to those shown in Figure 58, and then click **OK** to set the values.

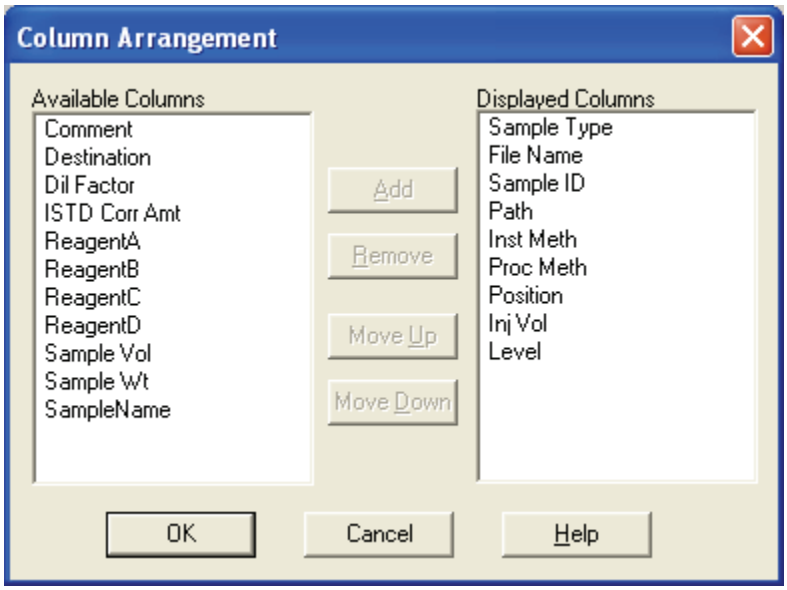
Figure 58. User Labels dialog box, showing the user-defined headings



3. Click the **Column Arrangement** button.

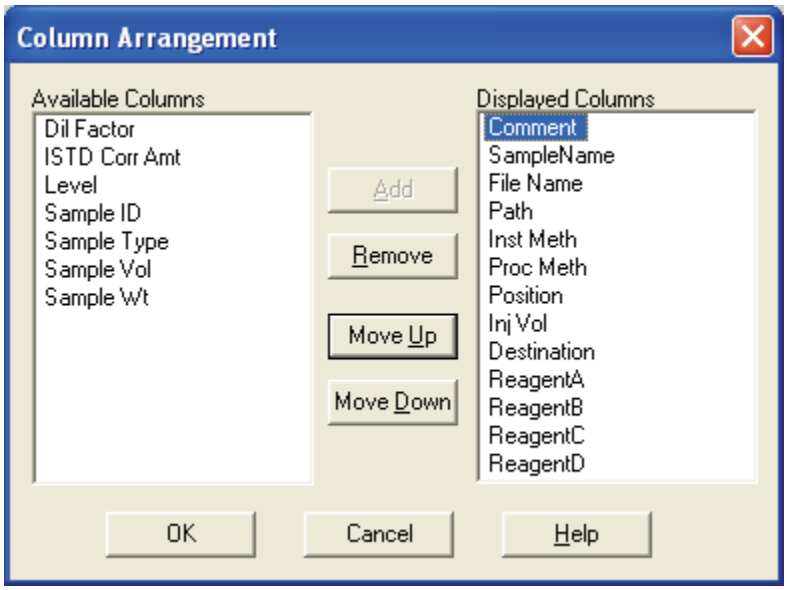
   The Column Arrangement dialog box appears as shown in Figure 59.

Figure 59. Column Arrangement dialog box



4. Use the Add, Remove, Move Up and Move Down buttons to change the Displayed Columns window to that shown in Figure 60.

Figure 60. Modified column arrangement

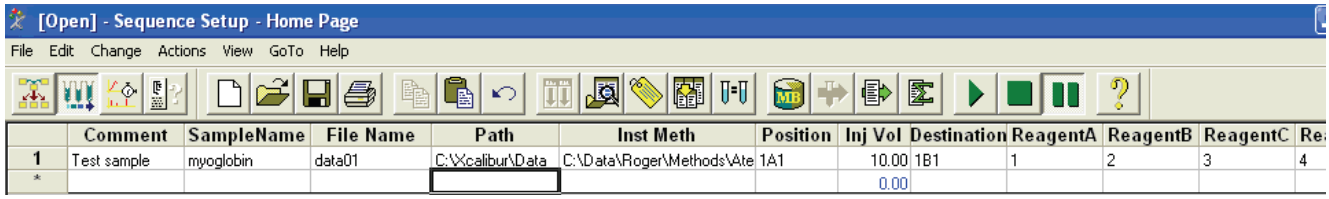


5. Click **OK** to exit the Column Arrangement dialog box and save the spreadsheet arrangement.

6. In the Destination, ReagentA, ReagentB, ReagentC, and ReagentD columns, type the location of the vials to indicate their position in the autosampler tray compartment as shown in Figure 61.

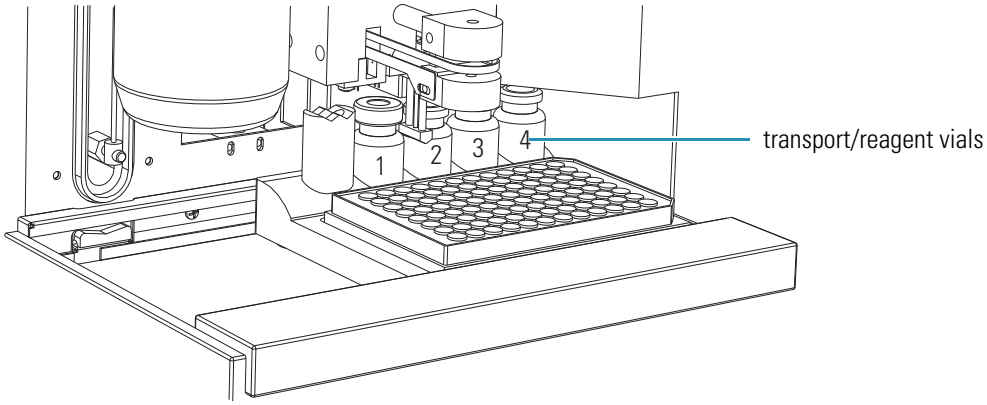
   From left to right, the reagent vial positions in the autosampler tray compartment are 1 through 4 as shown in Figure 62.

Figure 61. Modified sequence spreadsheet



	Comment	SampleName	File Name	Path	Inst Meth	Position	Inj Vol	Destination	ReagentA	ReagentB	ReagentC	Re
1	Test sample	myoglobin	data01	C:\Xcalibur\Data	C:\Data\Roger\Methods\Ate	1A1	10.00	1B1	1	2	3	4
*							0.00					

Figure 62. Position of reagent vials in the tray compartment



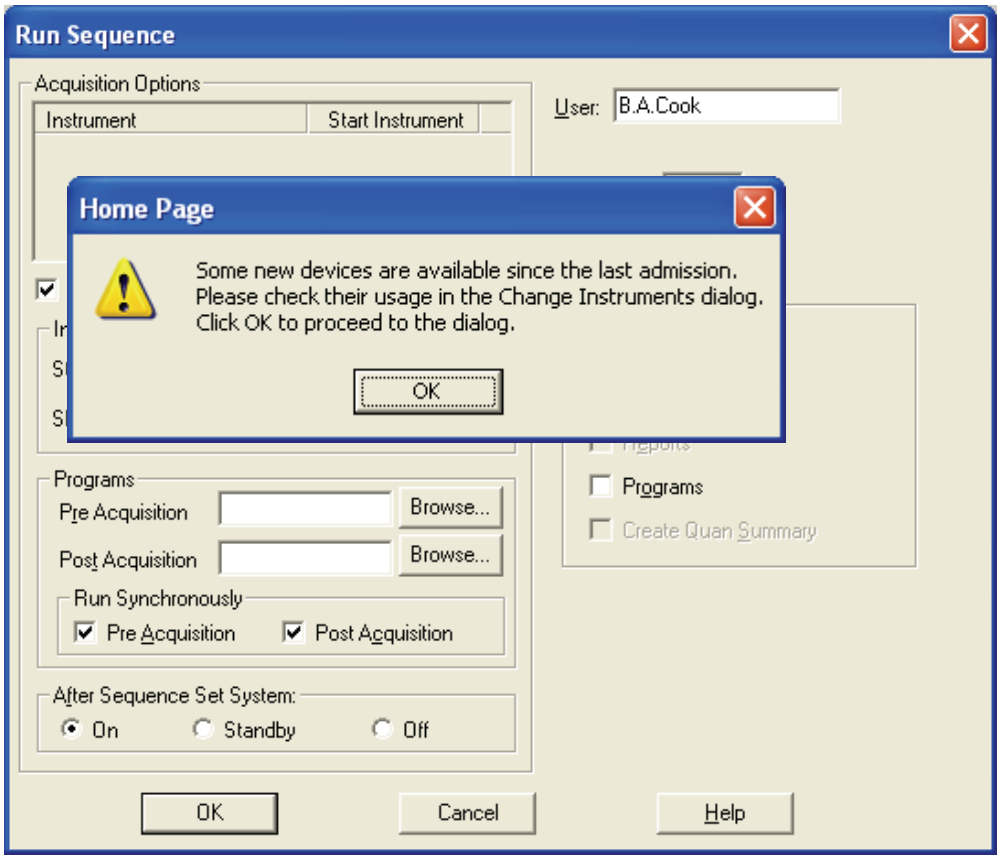
Starting a Sequence Run

❖ To start a sequence run

1. Check the status of the configured instruments.

2. Click the **Run Sequence** button.
3. Do one of the following:
   - If this is the first time you are running a sequence with your new instrument configuration, the Run Sequence dialog box along with a prompt to open the Change Instruments dialog box appears as shown in Figure 63. Go to step 4.
   - If you have previously run a sequence with your current instrument configuration, no prompt dialog box appears. In the Acquisition Options area, verify that no instrument is listed in the Start Instrument column, and then go to step 7.

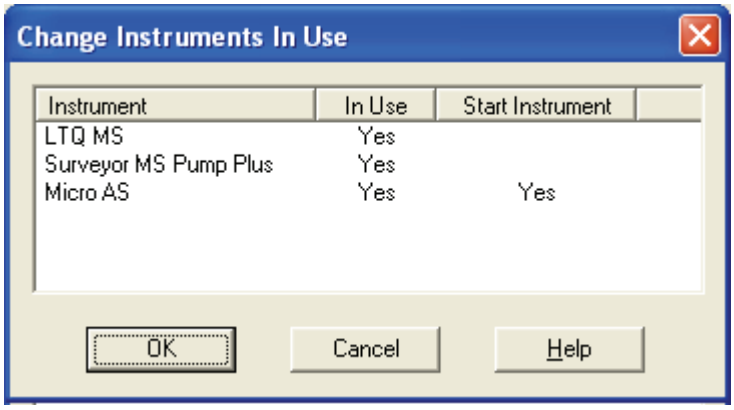
Figure 63. Run Sequence dialog box and Open Change Instruments dialog box prompt



4. Click **OK** in the prompt message dialog box.

As Figure 64 shows, the Change Instruments In Use dialog box appears, showing the Micro AS autosampler selected as the start instrument.

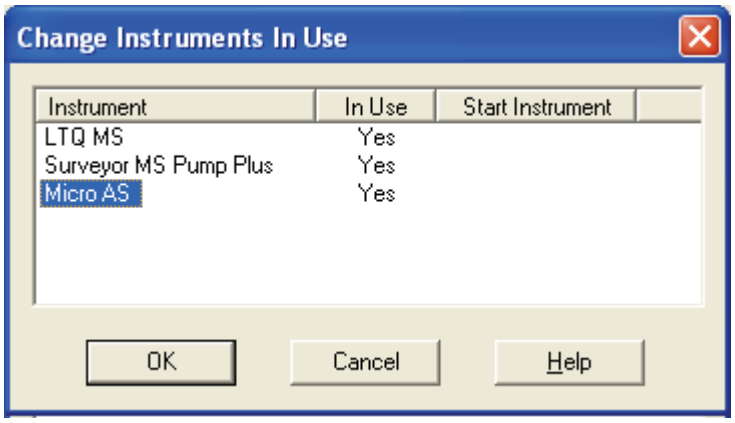
Figure 64. Change Instruments In Use dialog box, showing the Micro AS autosampler selected as the Start Instrument



5. In the Start Instrument column, click **Yes**.
6. As Figure 65 shows, Yes disappears from the Start Instrument column, which means that no instrument is specified as the start instrument.

Note By default, the configured autosampler is listed as the start instrument. If you do not ensure that no instrument is listed as the start instrument, the status of the Micro AS autosampler remains at Waiting for Download when you attempt to start a sequence run.

Figure 65. Change Instruments In Use dialog box, showing no start instrument



7. Make the appropriate selections in the Run Sequence dialog box and click **OK** to start the sequence run.

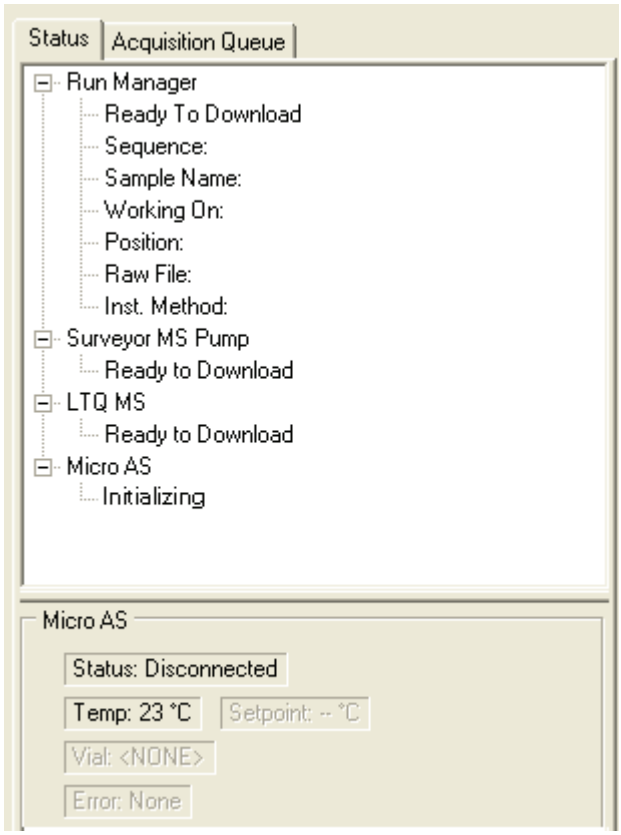
Recovering from an Initialization Error

Clicking the Stop Analysis button during a sequence run can occasionally cause an autosampler initialization error.

❖ To recover from an initialization error

1. Recycle the power to the Micro AS autosampler.
2. Turning the power switch off and then back on causes the autosampler to go through it start-up initialization process.

Figure 66. Micro AS Status page, showing the Micro AS status as Initializing



Test Procedures

This chapter describes some tests that you can use to check the performance of your Micro AS autosampler.

Contents

Syringe Volume Displacement Test

To check the syringe volume displacement, 20 µL of water is dispensed from a sample vial into a weighed destination vial.

❖ **To carry out the syringe volume displacement check**

1. Fill a sample vial with distilled water.
2. Weigh an empty destination vial.
3. Place the sample vial at position A01 and the destination vial at position A02 in the autosampler.
4. Program the autosampler with the mix method shown in Table 13 and the series shown in Table 14. Execute the series.
5. Weigh the destination vial after the series is complete. The difference in mass divided by the density of water (1 g/mL) is the volume dispensed by the syringe.

Table 13. Mix method for syringe volume displacement check

Step	Action	Speed	Height
1	Aspirate 25 µL Sample	2	05
2	Dispense 200 µL to Destination	3	03
3	End of mix method		

Table 14. Series for syringe volume displacement check

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

Tray Cooling Performance Test

❖ **To test the performance of the cooling tray on your autosampler**

1. Place a thermocouple on the bottom of the tray. Make sure that the contact between the tray and the thermocouple is firm.
2. Switch on the tray cooling and program a setpoint of 10 °C.
3. Wait at least 15 minutes for the Micro AS to equilibrate.
4. Read the temperature of the thermocouple.

The value must be within 2 °C of the programmed setpoint.

Loop Performance Test

❖ **To check that the sample loop volume falls within the specified volume range**

1. Disconnect the loop from the injection valve.
2. Remove all liquids from the loop with air.
3. Weigh the empty loop on an analytical balance.
4. Fill the loop, using a minimum of 2 times its volume of water.
5. Weigh the filled loop again.

The difference in mass between the filled loop and the empty loop is the mass of the capacity of the loop. The mass divided by the density of water (1 g/mL) gives the volume of the loop. The calculated volume should be within ± 10% of nominal volume of the sample loop.

Maintenance

This chapter contains procedures for routine maintenance of the Micro AS autosampler.

Contents

Replacing the Syringe

Replace the autosampler syringe if it becomes worn or leaks. Follow the procedure described in "Replacing the Syringe" on page 47 to replace the syringe.

Injection Valve Maintenance

The Micro AS autosampler is equipped with a Valco Cheminert™ C2-1346 injection valve. The material in this section is adapted from **Valco Technical Note 801**, Valco Instrument Co. Inc.

If you experience problems with your injection valve, first try cleaning the valve by flushing all lines with an appropriate solvent. If you cannot successfully clean the valve by flushing with solvent, follow the instructions below to disassemble the valve and clean or replace the rotor.

Note Do not disassemble the valve unless you have isolated the cause of your system malfunction to the valve. Refer to Chapter 9, "Troubleshooting," for more information on diagnosing problems with your system.

Valve Disassembly

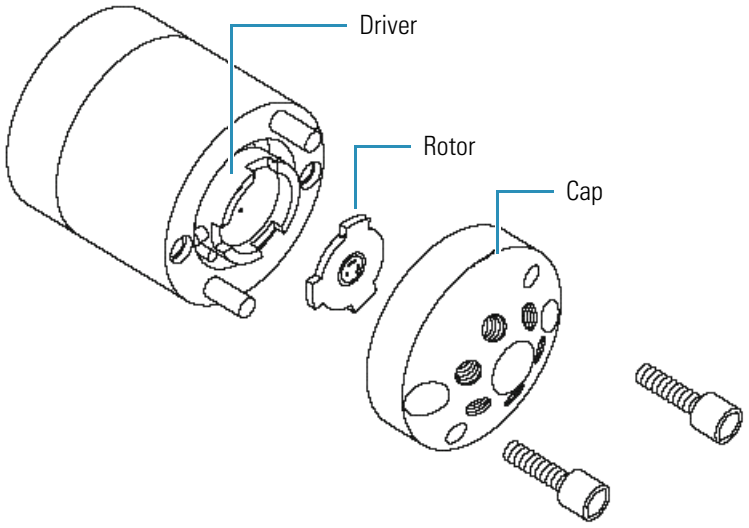
❖ To disassemble the valve

1. Use a 9/64 in. hex driver to remove the socket head screws that secure the cap on the valve. (See Figure 67.)
2. To ensure that the sealing surface of the cap is not damaged, either rest the cap on its outer face, or, if it is still connected to tubing, leave it suspended by the tubing.
3. With your fingers or a small tool, gently pry the rotor away from the driver.
4. Examine the rotor sealing surface for scratches. If scratches are visible to the naked eye, replace the rotor with a new one. If no scratches are visible, clean all valve parts with an appropriate solvent, taking care not to scratch any surfaces. You do not need to dry the rotor or other parts.

> **Tip** The most common valve problem in HPLC is the formation of buffer crystals in the valve, which can usually be removed by flushing with water.

Reassemble the valve as described in the next topic: Valve Reassembly.

Figure 67. Exploded view of injection valve



Valve Reassembly

❖ **To reassemble the injection valve**

1. Replace the rotor in the driver, making sure that the rotor sealing surface with its engraved flow passages is facing out. The pattern is asymmetrical to prevent improper placement.
2. Replace the cap. Insert the two socket-head screws and tighten them gently until both are snug. Do not overtighten the screws. The screws simply hold the assembly together and do not affect the sealing force, which is automatically set as the screws close the cap against the valve body.
3. Test the valve by pressurizing the system. If the valve does not hold pressure, contact your local Thermo Fisher Scientific service representative.

Replacing Fuses

Two fuses protect the Micro AS autosampler from damage due to current overload. If a power surge or other event causes these fuses to blow, you need to replace the fuses.

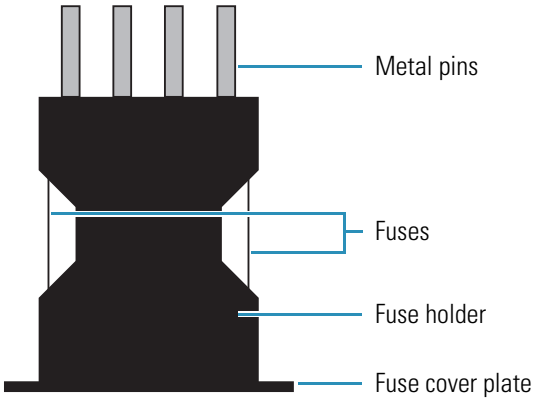
❖ **To replace the fuse**

1. Switch off the Micro AS autosampler and unplug the line power cord from the back of the autosampler.

CAUTION Do not attempt to change the fuses unless the autosampler is switched off and unplugged from power sources.

2. Insert a 3 mm (1/8 in.) or similar size flat-bladed screwdriver into the shallow slot at the top of the fuse cover plate. Pry gently outwards to dislodge the fuse assembly.
3. Remove the old fuses from the slots on either side of the fuse assembly. See Figure 68.

Figure 68. Schematic of fuse assembly



4. Insert new fuses into the slots on either side of the fuse assembly. Make sure that the fuses are firmly seated.

CAUTION Use only the correct fuses for your operating voltage. Refer to Appendix E, "Specifications," for more information on fuses.

5. Slide the fuse assembly into the autosampler until it clicks into place and the fuse cover plate is flush with the back of the autosampler. Ensure that the voltage selector is in the correct orientation for your operating voltage. Your operating voltage should be printed right side up at the bottom of the fuse cover plate.

Tip The fuse assembly slides easily into place if it is properly aligned in the socket. If you have difficulty reinserting the fuse assembly, shift it from side to side in the socket to find the correct alignment.

Troubleshooting

This chapter describes how to diagnose and troubleshoot problems with your Micro AS autosampler. In many cases, a physical fault in the autosampler causes a two-digit error code to be displayed on the front panel. If you encounter a problem in operating your autosampler, check the display for an error code and refer to "Error Codes" on page 85 to determine the nature of the problem.

If no error code is displayed, refer to "Start-up Problems" on page 92 or "Analytical Problems" on page 93, as appropriate, for additional troubleshooting information.

If you are unable to solve the problem using the procedures in this chapter, contact your local Thermo Fisher Scientific service representative.

Contents

Error Codes

If you attempt to enter invalid programming parameters, the Micro AS front panel displays an **error message**, with information on the allowed parameter ranges. If something goes wrong in the physical operation of the Micro AS, the front panel displays an **error code**. Press the **Start/Stop** key twice to clear the message, and try to repair the failure condition with the help of the explanation of the code concerned. Call your local Thermo Fisher Scientific service representative if the problem persists.

The following topics list the error codes:

- Vials
- Electronics
- Plate Holder

Injection Valve and ISS Unit

Table 15 lists the error codes related to the operation of the injection valve and ISS unit.

Table 15. Injection valve and ISS unit error codes

Error code	Description
ERROR 11	Injection valve is not in a valid position.
ERROR 12	The injection valve did not switch within 1.5 s.
ERROR 13	The switching time of the injection valve exceeds 500 ms.
ERROR 14	ISS-A valve is not in a valid position.
ERROR 15	The ISS-A valve did not switch within 1.5 s.
ERROR 17	ISS-B valve is not in a valid position.
ERROR 18	The ISS-B valve did not switch within 1.5 s.

Syringe Dispenser Unit

Table 16 lists the error codes for the syringe dispenser unit.

Table 16. Syringe dispenser unit error codes

Error code	Description
ERROR 21	The syringe valve did not switch.
ERROR 22	The syringe did not reach the home position in time.
ERROR 23	The syringe spindle did not make the correct number of rotations.
ERROR 24	The spindle does not rotate.
ERROR 25	The syringe valve did not find a valid position.

Injection Needle Unit

Table 17 lists the error codes for the injection needle unit.

Table 17. Injection needle unit error codes

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

Plate

Table 18 lists error codes related to plates.

Table 18. Plate error codes

Error code	Description
ERROR 58	Not enough plates in right lift to execute run of series.
ERROR 59	Missing plate.

Vials

Table 19 lists error codes for vials.

Table 19. Vials error codes

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

Electronics

Table 20 lists error codes related to the autosampler electronics.

Table 20. Electronics error codes

Error code	Description
ERROR 71	Flexprint of the sample needle is not connected.
ERROR 72	Invalid configuration of the Micro AS, a PCB is missing.
ERROR 73	Current limit of the external I/O exceeded.
ERROR 75	Error occurred during initialization, the Micro AS cannot start.

Plate Holder

Table 21 lists error codes related to the plate holder.

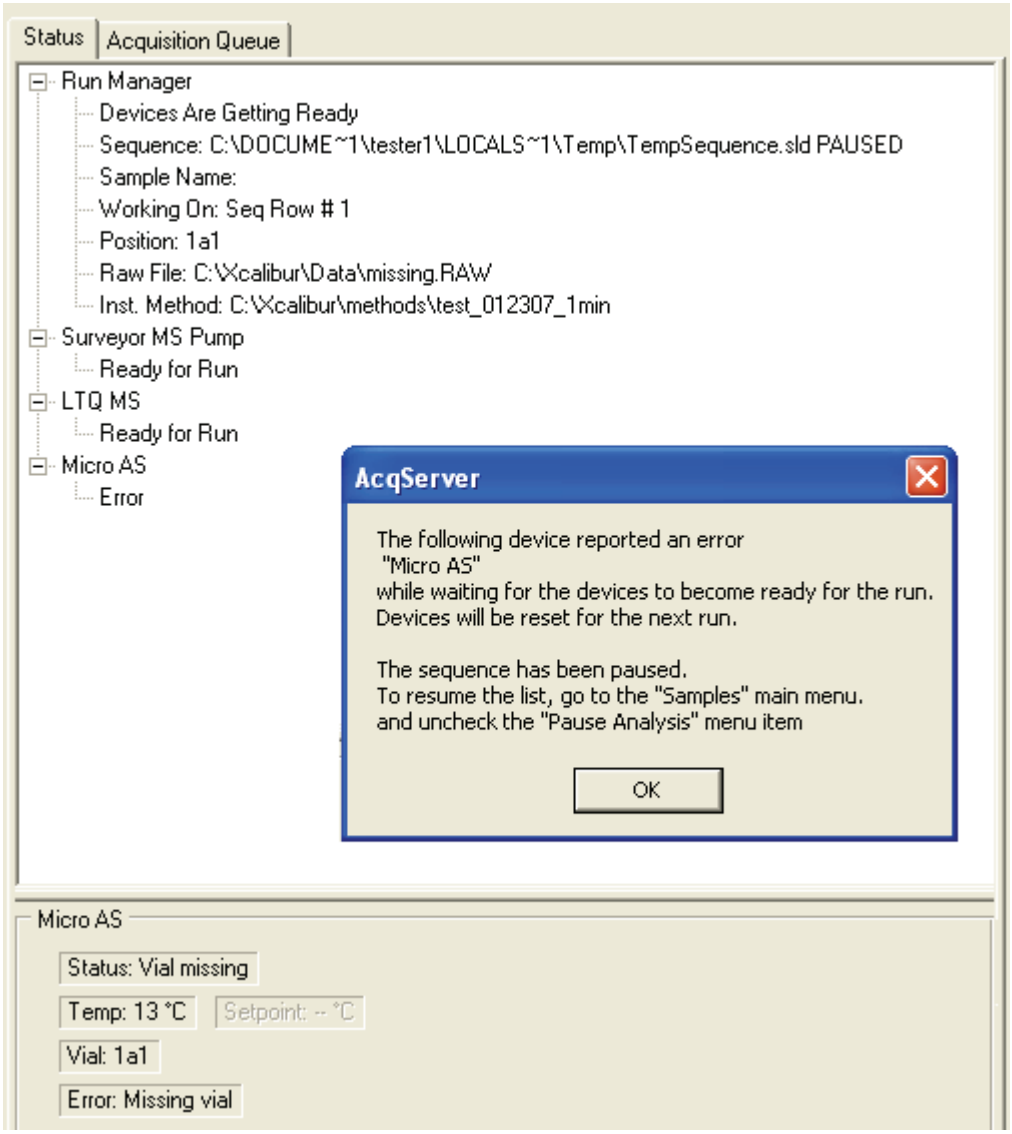
Table 21. Plate holder error codes

Error code	Description
ERROR 90	Plate home time-out, plate did not reach the home position (home error).
ERROR 91	Plate did not reach or leave the home position during run.
ERROR 92	Plate holder missing.
ERROR 93	Dirt on the plate holder.

Recovering From a Missing Vial Error

When you operate the Micro AS using the Xcalibur data system, the autosampler pauses and displays the flashing error code 60 in the LCD display if it encounters a missing vial during the run. Xcalibur reports that the Micro AS has a 'Missing vial' error as shown in Figure 69.

Figure 69. Status page, showing the 'Missing vial' error message



❖ To recover from this error and resume the run

1. On the front panel, press the second soft-function key from the left under the LCD display.

   The instrument resets.

2. Place a vial in the empty position in the sample tray. This removes the source of the error.

3. Press **Menu**, and then press SERIAL to place the autosampler into the serial mode.

   The status for the Micro AS in Xcalibur returns to 'Ready to download', but the status for the Surveyor MS Pump remains at 'Ready to run'.

4. In the Acq Server dialog box, click **OK**.

5. Right-click the Surveyor MS Pump listing in the Status directory and choose **Turn Device On**.

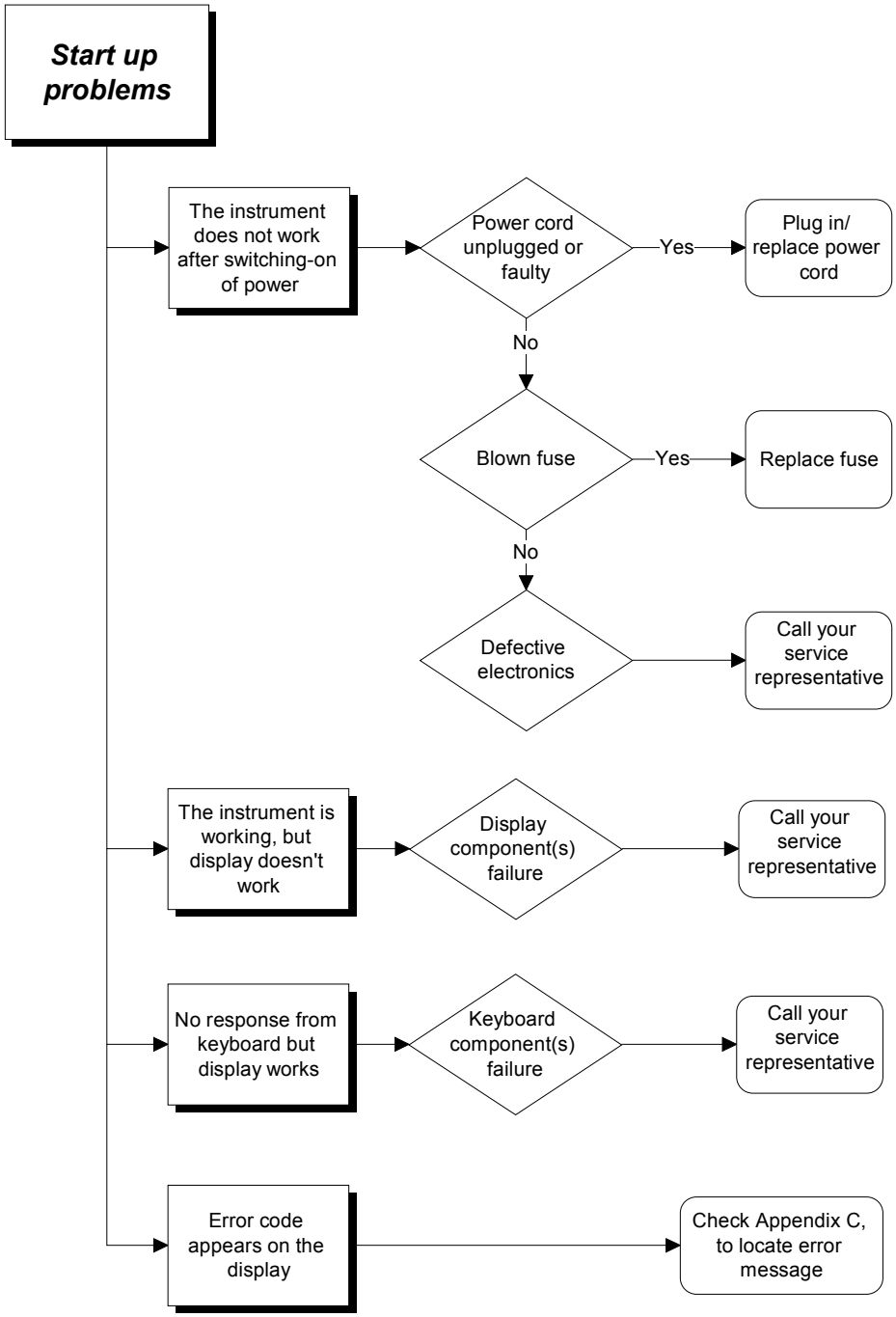
   The status for the Surveyor MS Pump returns to 'Ready to download'.

6. In the Xcalibur toolbar, click the **Pause/Resume** button to resume the run.

Start-up Problems

If your Micro AS autosampler does not start up correctly, consult Figure 70 for troubleshooting information.

Figure 70. Troubleshooting flowchart for start-up problems



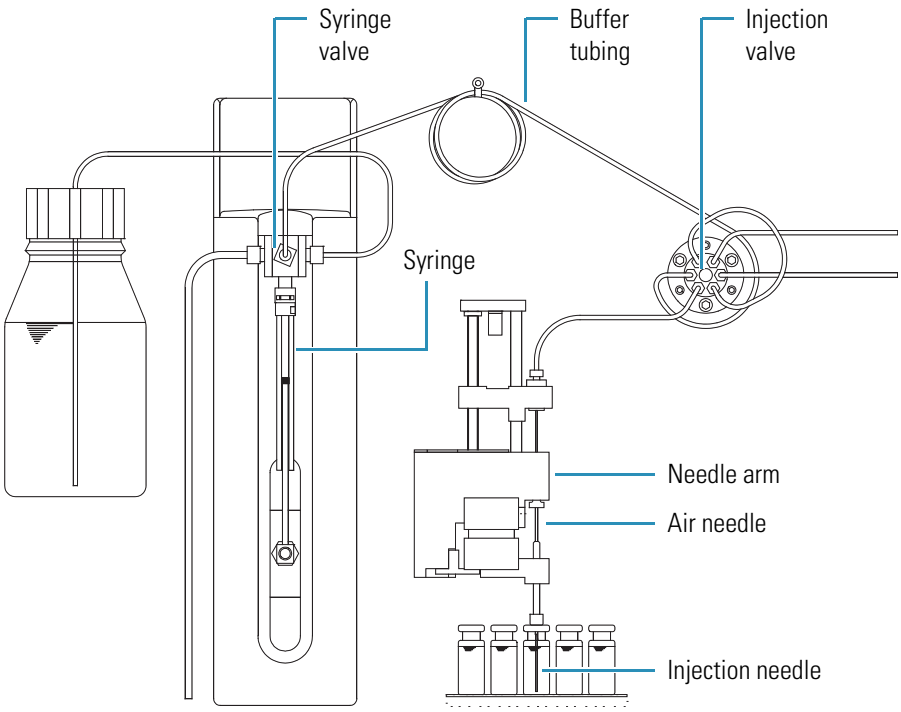
Analytical Problems

If you experience analytical problems, you will have to determine whether they are caused by the autosampler or by the rest of the HPLC system. Replace the autosampler with a manual injection valve, and perform several manual full loop injections. If these produce good results, then the autosampler is faulty. If not, then another component of the HPLC system is at fault.

Note Analytical problems might be caused by external influences, such as temperature, use of light-sensitive samples, and so on. For this reason, it is important to troubleshoot analytical problems with the autosampler using samples and System Menu settings that are known to give acceptable analytical performance.

Figure 71 illustrates the components of the autosampler that should be checked when troubleshooting an analytical problem.

Figure 71. Autosampler schematic, showing components to be checked in troubleshooting analytical problems



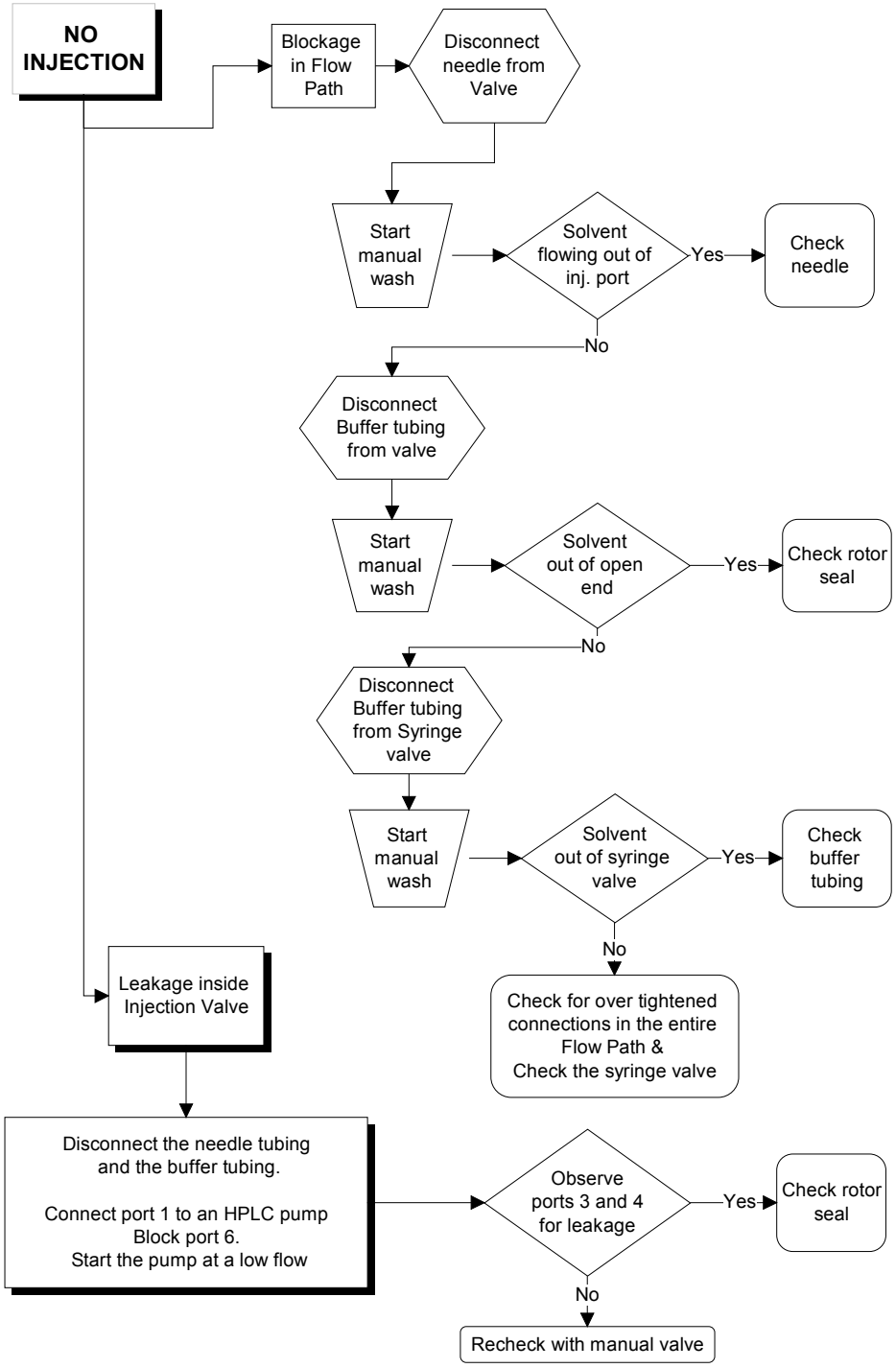
This section contains topics specifically discussing the troubleshooting of:

- Injection Problems
- Reproducibility Problems

Injection Problems

Figure 72 illustrates a flowchart for troubleshooting injection problems.

Figure 72. Flowchart for troubleshooting injection problems



Reproducibility Problems

This topic describes what to do if you have reproducibility problems with your Micro AS autosampler. It contains the following subtopics:

Removing an Air Bubble From the Syringe

The most common cause of reproducibility problems with the Micro AS autosampler is a trapped air bubble in the syringe.

❖ To diagnose and fix this problem

1. On the front keypad of the autosampler, press EXIT to exit serial mode and enter the Ready Menu.
2. Press SYR END. The syringe will move to its end position.

   If there is an air bubble trapped in the syringe, it should be clearly visible when the syringe is in the end position.
3. First, try to expel the air bubble by running a wash:
   a. Press SYR HOME to return the syringe to the home position.
   b. Press WASH to activate the wash procedure.
   c. Let the wash procedure run until the syringe has been filled and emptied several times. Press **Start/Stop** to stop the wash.
   d. Check the syringe again for air bubbles. If there is still air in the syringe, go to step 4. If the air bubble has been expelled, go to step 11.
4. Remove the syringe from the autosampler:
   a. Press SYR END. The syringe will move to its end position.
   b. Lift the cover.
   c. If necessary, remove the wash solvent bottle and holder to allow easier access to the syringe. To do this, pull on the wash solvent bottle holder to slide the tabs on the holder out of the slots in the autosampler.
   d. Unscrew the syringe from the autosampler by turning clockwise.
   e. Pull the bottom of the syringe towards you and slide the bottom of the syringe handle off of the peg on the autosampler.

5. Hold the syringe upright (with the syringe opening pointing upwards). Gently tap or flick the syringe with a finger to dislodge air bubbles and allow them to escape from the syringe.

   If you cannot dislodge the air bubble using this method, go to step 6. If the air bubble has been expelled, go to step 8.

6. Fill a small (~50 mL) beaker with wash solvent.

7. Submerge the syringe opening in the wash solvent in the beaker. Rapidly push down and draw up the syringe plunger. Repeat until the air bubble is expelled from the syringe.

8. Reinstall the syringe on the autosampler:

   a. Fit the hole in the end of the syringe handle over the peg on the autosampler.

   b. Screw the top of the syringe to the autosampler by turning counterclockwise.

   c. Close the autosampler cover.

   d. Press SYR HOME to return the syringe to the home position.

9. If you removed the wash solvent bottle earlier, reinstall it. Refer to "Installing the Wash Solvent Bottle" on page 44 for more information on installing the wash solvent bottle.

10. Execute a wash:

    a. Press WASH to start the wash procedure.

    b. Let the wash procedure run until the syringe has been filled and emptied several times. Press **Start/Stop** to stop the wash.

11. Press **Menu**, then press SERIAL to put the autosampler back into serial mode.

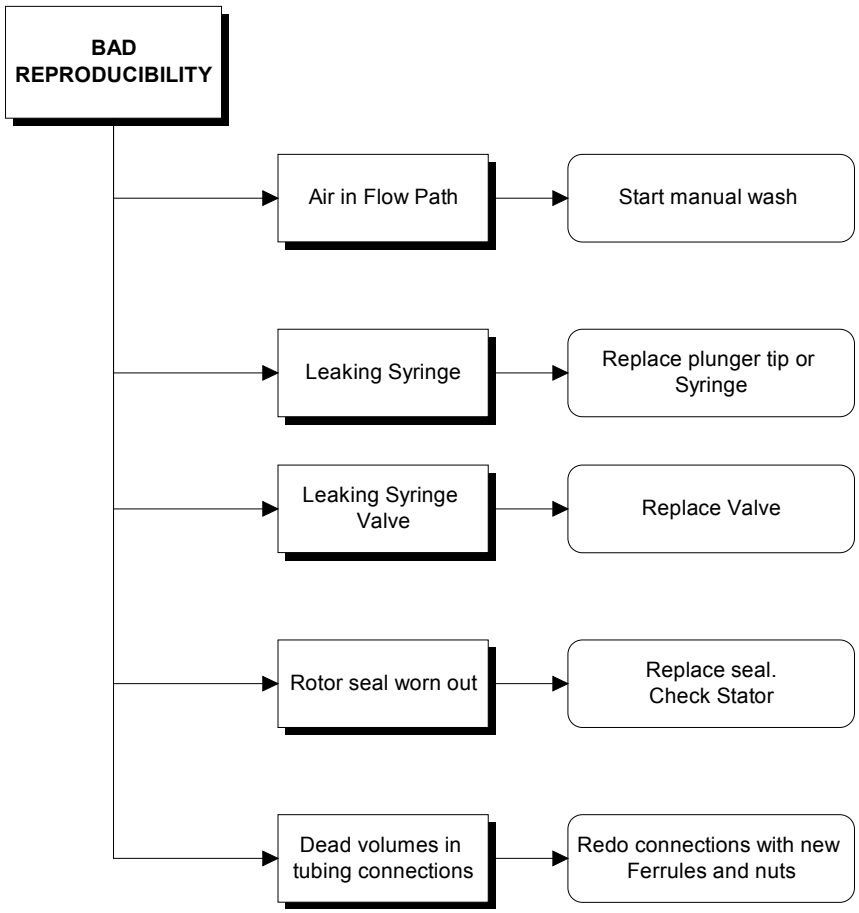
Note The autosampler must be placed into serial mode before you attempt to operate the autosampler using the data system control software. The data system computer will not be able to establish communication with the autosampler unless the autosampler is in serial mode.

Place the autosampler in serial mode by pressing the SERIAL soft-function key in the Ready Menu.

Reproducibility Troubleshooting Chart

Figure 73 illustrates a flowchart for troubleshooting additional reproducibility problems.

Figure 73. Flowchart for troubleshooting reproducibility problems



Parts and Accessories

This chapter contains a list of parts and accessories that can be ordered from your local Thermo Fisher Scientific service representative.

Contents

Parts and Accessories Kit

The Micro AS autosampler is shipped with a parts and accessories kit (P/N 00960-01-00010) that includes the parts listed in Table 22.

Table 22. Parts and accessories that are shipped with the Micro AS (Sheet 1 of 2)

Quantity	Description
1	48-vial adapter
1	Injection marker cable
1	2.4 µL biocompatible fused silica needle
1	5.0 µL biocompatible fused silica needle
1	0.25 in. female Luerlock fitting
1	3-stranded networking cable
2	Glass fuses, UL, ¼ in. × 1¼ in.
4	10 mL vials, 20 mm × 47 mm
4	Septa for vials
4	Caps for vials
1	Masterblock™ U 96-well polypropylene plate
1	96-well polystyrene plate
1	Capmat for Masterblock 96-well plate
1	10 µL PEEK injection loop

Table 22. Parts and accessories that are shipped with the Micro AS (Sheet 2 of 2)

Quantity	Description
1	100 µL Luer™ lock syringe
1.2 m	Silicone tubing, ID 7.0 mm, OD 10.0 mm

Additional Replaceable Parts

The following additional replacement parts are available from your local Thermo Fisher Scientific service representative:

Syringe, 25 µL. 00950-01-00002
Seal, rotor, C2-1346 injection valve. 00950-01-00003
Needle, fused silica, 2.4 µL, biocompatible 00950-01-00004
Valve, syringe 00950-01-00005
Air prepuncturing needle. 00950-01-00006
Opto interruptor. 00950-01-00007
Stator, C2-1346 injection valve 00950-01-00008
Cable, inject marker 00950-01-00009
Stepper motor, 1.05 A. 00950-01-00010
Rubber foot kit (6 pieces) 00950-01-00011
Stepper motor, 2.2 A. 00950-01-00012
Tubing, syringe waste 00950-01-00013
Tubing, buffer, 50 µL, PEEK. 00950-01-00014
Transport nut M5 00950-01-00015
Sealing ring M5 (10 pack). 00950-01-00016
Needle body 00950-01-00017
Waste outlet 00950-01-00018
Flex PCB with sample needle motor 00960-01-00006
Plate holder assembly, cooler 00960-01-00008
Peltier assembly. 00960-01-00009

Introduction to Keypad Operation

This chapter describes how to operate and program the Micro AS autosampler from the front keypad.

When you switch on the Micro AS autosampler, it defaults to the serial mode of operation. In serial mode, the autosampler is controlled by the data system computer and not from the front keypad.

Before trying any of the programming examples in this chapter or performing other operations involving control of the autosampler from the front keypad, you must place the autosampler into manual mode. Press EXIT on the front keypad to exit serial mode and enter manual mode.

When you want to return to controlling the autosampler from the data system computer, you must put the autosampler back into serial mode. The data system computer cannot establish communication with the autosampler unless the autosampler is in serial mode. From the Ready Menu, press SERIAL to put the autosampler into serial mode.

Contents

Menus

The software of the Micro AS is menu-driven. The most important menus are:

- **Ready Menu**: This menu appears after the Micro AS has been switched on and the Serial mode has been exited. It offers general options for methods management and communication with other instruments.
- **System Menu**: This menu appears after you press the **System** key in the keypad. It offers general options for entering settings for an analytical run. The settings programmed into the System Menu affect the options that are displayed in other menus. To keep other menus as concise as possible, enable only the options you need in the System Menu.
- **Methods Menu**: This menu appears after you press the **Methods** key in the keypad. The menu allows you to program methods to be used in analytical runs.
- **Series Menu**: This menu appears after you press the **Series** key in the keypad. The menu allows you to define a series. A series is a program that links one or more methods to a set of sample wells.

Use the following **keys** to navigate through the menus:

- **E**: (Enter) To confirm a choice or to select a choice made in a screen, or to step through menu lines
- **Cursor keys**: To change values in a field or to move to a different field in a screen
- **Escape**: To return to a previous menu
- **CL**: (Clear) To remove a value from a field and enter NONE or DEFAULT
- **Soft function keys**: To go to sub-menus

If MENU or MN is displayed in the top right hand corner of the screen, you can press the **Menu** key in the keypad to display more options offered by the menu. Refer to "Keypad and Display of the Micro AS" on page 7 for an overview of all the keys in the keypad.

Recommended Working Order

After you have determined what type of analytical run you want to perform, the most convenient working order for the Micro AS is:

1. Make any necessary changes to settings in the System Menu.
2. Program a method for the analyses you want to perform in the Methods Menu.
3. Define a series and link a programmed method to a range of wells in the Series Menu.
4. Execute the series.

You can use a different order; however, you should keep in mind that settings entered in the System Menu determine which options appear in the Methods and Series menus. Refer to Appendix B, "Keypad Menu Reference," for more information on specific items in menus and the way they influence the other menus. Refer to "Programming Examples" on page 105 for a number of examples illustrating this working order.

Types of Methods and Links to Series

The Micro AS offers the following types of methods for different parts of the sample handling routine:

- **Injection** method: Contains information on the injection routine, flush volume and analysis time.
- **Wash** method: Describes a wash volume and when a wash is executed.
- **Mix** method: A pre-injection method in which additional sample handling can be performed (for example, a pre-column derivatization).
- **Timebase** method: A post-injection method with which outputs to other devices (for example, an integrator or pump) and switching of the ISS valve are controlled.
- **User program**: Offers the option to program sequences of all actions that can be executed by the Micro AS in separate steps.

Each programmed method is assigned a number. The Micro AS offers the option to store a combination of defined methods in a **template**. A template is also identified by a number.

Methods must be linked to series before they can be used. The following options are offered by the Micro AS:

- You can assign an individual **method** to a series. Mix, injection, wash, and timebase methods can be linked to wells in a series.
- You can assign a **template** to a series. A combination of various programmed methods (mix, injection, wash, timebase) can be defined in a template. The template is linked to a range of wells in a series. In this way all steps in an analytical run are stored together.
- You can assign a **user program** to a series. You can program the user program with a sequence containing any of the available actions that the autosampler can perform in the order you choose.

Executing a Series

Execution of a series is only possible if you have programmed a method and defined a series for the samples you wish to analyze. Series are not stored in battery backup and exist only for as long as the Micro AS is switched on.

❖ To execute one or more series

1. Press **Start/Stop**.
2. Enter the number of the first series to perform and the number of the last series to perform.
3. Press START to start the actual analytical run. The Micro AS will begin to execute the series you have defined. Series are executed in numerical order. Undefined series are skipped.

After the Micro AS has executed the run, the Ready Menu appears again.

It is possible to program series and methods during a run. Press **Series** or **Methods**; the options offered in the menus are identical to those offered when the Micro AS is idle.

If a series or method is changed, the new values will become active the next time the Micro AS starts a series. The series currently running are not affected by the changes.

Executing a Series in Remote Control

❖ To execute a series from remote control

1. Press **Start/Stop**.
2. Enter the numbers of the first and the last series to be performed.
3. Press REMOTE to enter the remote control mode. The Micro AS can now be controlled by another device using the Next injection input and the Next well input. To indicate that remote control is active, an “r” is displayed in the bottom left corner of the display during execution of the series. At the end of the series the message “Series completed via remote control” is displayed.
4. Press **Escape** to return to the Ready Menu.

Refer to Chapter 3, “I/O Connections,” for more information on remote control.

Programming Examples

This section presents a number of examples of programs that can be created and run from the front panel of the Micro AS autosampler. You might find it helpful to do these examples to learn how to work with the autosampler. This section does not provide a description of all autosampler commands. Refer to Appendix B, "Keypad Menu Reference."

These examples can be executed after the Micro AS has been installed in accordance with Chapter 2, "Installation," and after all items described in Chapter 5, "Preparing for Use," have been correctly set up.

The following examples are described in this section:

- Example 1. A 10 µL Partial Loopfill Injection
- Example 2. A 3 × 1µL Injection With µL Pick-up and Wash Between Injections
- Example 3. A 1:10 Dilution Followed by Injection
- Example 4. Defining a Template and Adding a Protection Code

Example 1. A 10 µL Partial Loopfill Injection

This example illustrates how to perform a single partial loopfill injection. For this example it is assumed that a loop of 20 µL, needle tubing of 2.4 µL and a syringe of 25 µL have been installed.

❖ To execute this example

1. Switch on the power to the autosampler and wait until the Ready Menu appears.
2. Set the System settings as described in Table 23. All settings not specified should be set to the default values.

Table 23. System settings for Example 1 (Sheet 1 of 2)

Keys pressed	Description
System	Enters the System Menu
MICRO **E**	Selects Micro mode
GENERAL **E**	Enters the General Menu
[2000] **E**	Defines the volume of the installed loop
[024] **E**	Defines the volume of the needle tubing
NORMAL **E**	Sets the syringe speed to normal
[05] **E**	Sets the sample needle height to 5 mm
YES **E**	Enables use of air segment
NO **E**	Switches off headspace pressure

Table 23. System settings for Example 1 (Sheet 2 of 2)

Keys pressed	Description
Escape	Returns to the System Menu
PLATES **E**	Enters the Plates Menu
96-LOW	Defines the type of plate to be used
IN ROWS	Defines the processing order of wells
Escape Escape	Returns to the Ready Menu

3. Program the injection method as described in Table 24.

Table 24. Injection Method Program for Example 1

Keys pressed	Description
Methods	Enters the Methods Menu
INJECTION [01] **E**	Selects injection method number 1
PARTIAL **E**	Selects partial loopfill injection method
[100] **E**	Defines an analysis time of 1 minute
[050] **E**	Defines a flush volume of 5 µL
[1] **E**	Defines the number of injections per well
[1000] **E**	Sets the injection volume at 10.0 µL
Escape Escape	Returns to the Ready Menu

4. Program a series as described in Table 25.

Table 25. Series Program for Example 1

Keys pressed	Description
Series	Enters the Series Menu
[01] **E**	Defines the Series number
[01] **E**	Defines the injection method number
CL E	Enters <NONE> for wash method
ROW A [01] **E**	Defines location of the first sample well
ROW A [01] **E**	Defines location of the last sample well
Escape	Returns to the Ready Menu

5. Place a sample in position A 01 of the plate.
6. Run the series:
   a. Press **Start/Stop** to start the autosampler.
   b. Press [01] **E** to start execution with series number 1.

c. Press [01] **E** to end execution after series number 1.

d. Press START to start the analytical run.

The Micro AS autosampler locates well A 01 and performs a 10 µL partial loopfill injection. The autosampler display indicates the instrument status (Checking tray, Flushing, Loopfill, Running, Rinse buffer, Running). The display also indicates the number of the defined series (01), the method number (01) and the well on which the analysis is performed (A 01).

At the end of the defined analysis time the Ready Menu is displayed again to indicate that the Micro AS is ready for the next analytical run.

Example 2. A 3 × 1µL Injection With µL Pick-up and Wash Between Injections

This example makes use of the µL pick-up injection method rather than the partial loopfill injection method used in the previous example. It also illustrates how to carry out multiple injections from the same well, and how to program a wash step between injections.

❖ To execute this example

1. From the Ready Menu, program the System settings as illustrated in Table 26.

Table 26. System settings for Example 2

Keys pressed	Description
System	Enters the System Menu
MICRO E	Selects Micro mode
GENERAL **E**	Enters the General Menu
E until Air segment appears	Selects the Air segment field
NO **E**	Switches off the air segment
Escape	Returns to the System Menu
PLATES **E**	Enters the Plates Menu
E E	Selects the transport vials field
[1] **E**	Defines the position of the first transport vial
[1]	Defines the position of the last transport vial
Escape Escape	Returns to the Ready Menu

2. Program injection and wash methods as described in Table 27.

Table 27. Methods program for Example 2 (Sheet 1 of 2)

Keys pressed	Description
Methods	Enters the Methods Menu
INJECTION [02] **E**	Selects injection method number 02

Table 27. Methods program for Example 2 (Sheet 2 of 2)

Keys pressed	Description
PICK-UP **E**	Selects the injection mode for this method
[100] **E**	Defines the analysis time as 1 minute
[3] **E**	Defines the number of injections per well
[100] **E**	Defines a volume of 1.0 µL for 1st injection
[100] **E**	Defines a volume of 1.0 µL for 2nd injection
[100] **E**	Defines a volume of 1.0 µL for 3rd injection
Escape	Returns to the Methods Menu
WASH	Enters the Wash Menu
[01] **E**	Selects wash method number 01
INJECTION **E**	Selects wash between injections
[300]	Defines wash volume
Escape Escape	Returns to the Ready Menu

3. Program the series as described in Table 28.

Table 28. Series program for Example 2

Keys pressed	Description
Series	Enters the Series Menu
[01] **E**	Defines the series number
[02] **E**	Defines the injection method for this series
[01] **E**	Defines the Wash method for this series
ROW A [01] **E**	Defines the location of the first sample well
ROW A [01] **E**	Defines the location of the last sample well
Escape	Returns to the Ready Menu

4. Place a vial containing transport solvent (mobile phase) in transport vial position 1 (left). Make sure the transport vial is filled before starting the run.
5. Place a 96 well plate with sample in well A1 on the plate holder.
6. Run the series:
   a. Press **Start/Stop** to start the autosampler.
   b. Press [01] **E** to start execution with series number 1.
   c. Press [01] **E** to end execution after series number 1.
   d. Press START to start the analytical run.

At the end of the defined analysis time the Ready Menu appears again to indicate that the Micro AS is ready for the following next run.

Example 3. A 1:10 Dilution Followed by Injection

This example illustrates how to program a Mix method to carry out a 1:10 dilution followed by a µL pick-up injection of the diluted sample.

❖ To execute this example

1. From the Ready Menu, program the System settings as described in Table 29.

Table 29. System settings for Example 3

Keys pressed	Description
System	Enters the System Menu
MICRO **E**	Selects Micro mode
USAGE **E**	Enters the Usage Menu
E E	Selects the Mix field
ENABLED	Enables use of mix methods
Escape	Returns to the System Menu
PLATES **E**	Enters the Plates Menu
96-LOW	Selects 96 well plate
IN COLUMNS	Selects processing in columns (A1, B1, and so on)
[1] **E**	Defines the position of the first transport vial
[1]	Defines the position of the last transport vial
Escape Escape	Returns to the Ready Menu

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

2. Program the injection method as described in Table 30.

Table 30. Injection method program for Example 3 (Sheet 1 of 2)

Keys pressed	Description
Methods	Enters the Methods Menu
INJECTION [03] **E**	Enters the Injection Menu
PICKUP **E**	Selects partial loopfill injection mode
[100] **E**	Defines the analysis time
[3] **E**	Defines the number of injections per well

Table 30. Injection method program for Example 3 (Sheet 2 of 2)

Keys pressed	Description
[100] **E**	Enters the injection volume of 1. 0 µL for 1st injection
[100] **E**	Enters the injection volume of 1. 0 µL for 2nd injection
[100] **E**	Enters the injection volume of 1. 0 µL for 3rd injection
Escape	Returns to the Methods Menu

3. Program the mix method as described in Table 31.

Table 31. Mix method for Example 3 (Sheet 1 of 2)

Keys pressed	Description
MIX	Enters the Mix Menu
[1] **E**	Defines Mix method number 1
INSERT	Inserts mix method step 1
ASPIRATE [200] <AIR> **E**	Aspirates an air segment of 2.0 µL
INSERT	Inserts mix method step 2
ASPIRATE [2000] **Menu** REAG-A **E**	Aspirates 20 µL from Reagent A vial
INSERT	Inserts mix method step 3
DISPENSE [1800] **E**	Dispenses 18.0 µL to destination well
INSERT	Inserts mix method step 4
DISPENSE [400] WASTE → [5] **E**	Dispenses 4.0 µL to waste
INSERT	Inserts mix method step 5
REPEAT [1] ← [4] **E**	Repeats the last four steps once
INSERT	Inserts mix method step 6
ASPIRATE [200] AIR **E**	Aspirates an air segment of 2.0 µL
INSERT	Inserts mix method step 7
ASPIRATE [600] SAMPLE **E**	Aspirates 6.0 µL of sample
INSERT	Inserts mix method step 8
DISPENSE [400] **E**	Dispenses 4.0 µL to destination well
INSERT	Inserts mix method step 8
DISPENSE [500] WASTE → [5] **E**	Dispenses 5.0 µL to waste
INSERT	Inserts mix method step 10

Table 31. Mix method for Example 3 (Sheet 2 of 2)

Keys pressed	Description
ASPIRATE [500] AIR **E**	Aspirates an air segment of 5.0 µL
INSERT	Inserts mix method step 11
ASPIRATE [2000] DESTINATION → [3] **E**	Aspirates 20.0 µL from the destination well with syringe speed 3
INSERT	Inserts mix method step 12
DISPENSE [2000] → [9] **E**	Dispenses 20.0 µL to the destination well with syringe speed 9
INSERT	Inserts mix method step 13
REPEAT [3]	Repeats the last 2 steps 3 times
Escape Escape	Returns to the Ready Menu

4. Program the series as described in Table 32.

Table 32. Series program for Example 3

Keys pressed	Description
Series	Enters the Series Menu
[01] **E**	Defines series number 1
[01] **E**	Selects Mix method number 1 for this series
[03] **E**	Selects Injection method number 3
CL E	Selects NONE for wash method
ROW A [1] **E**	Defines location of first sample well
ROW A [1] **E**	Defines location of last sample well
ROW B [1] **E**	Defines location of first destination well
[1] **E**	Defines position of Reagent A
Escape	Returns to the Ready Menu

5. Load a sample plate with sample in position A 01. Position B 01 is used as an empty sample well.
6. Place a filled reagent vial in position 1 of the reagent/transport vial slots. Make sure that the reagent vial is filled correctly before starting a new series.
7. Run the series:
   a. Press **Start/Stop** to start the autosampler.
   b. Press [01] **E** to start execution with series number 1.

c. Press [01] **E** to end execution after series number 1.

d. Press START to start the analytical run.

When the run is started, the Micro AS transports 18 µL of Reagent A to the destination well (B 01) twice. Then the autosampler adds 4 µL of sample to the destination well, and mixes the sample three times. After mixing, the autosampler performs a 1.0 µL injection.

Example 4. Defining a Template and Adding a Protection Code

This examples illustrates how to incorporate the injection method (02) and wash method (01) defined in Example 2 into a template and how to add a protection code to the methods. When a protection code is enabled, you cannot edit any methods without first entering the code.

❖ To execute this example

1. From the Ready Menu, program the system settings as shown in Table 33.

   Table 33. System settings for Example 4

Keys pressed	Description
System	Enters the System Menu
USAGE **E**	Enters the Usage Menu
[123456] **E**	Enters a 6-digit code (memorize or record this code!)
E	Selects the mix methods field
DISABLED	Turns off use of mix methods
E E	Selects the template field
ENABLED	Enables templates
Escape Escape	Returns to the Ready Menu

   After use of templates has been enabled the message "ALL SERIES DEFAULT" appears. You need to redefine the series because the System settings have changed.

2. Select the methods to be incorporated into the template as described in Table 34.

   Note The template used in this example incorporates the injection method and the wash method programmed in "Example 2. A 3 × 1µL Injection With µL Pick-up and Wash Between Injections" on page 107. If you have deleted or change these methods, refer to the above section and reprogram the injection method and wash method before continuing.

Table 34. Template for Example 4

Keys pressed	Description
Methods	Enters the Methods Menu
[123456] **E**	Enters the methods protection code
TEMPLATE	Enters the Template Menu
[01] **E**	Defines the number for the template
[02] **E**	Defines the injection method for this template
[01]	Defines the wash method for this template
Escape Escape	Returns to the Ready Menu

3. Program the series for the template as described in Table 35.

Table 35. Series Program for Example 4

Keys pressed	Description
Series	Enters the Series Menu
[01] **E**	Defines the Series number
[01] **E**	Defines the Template method number
ROW A [01] **E**	Defines the first sample well
ROW B [01] **E**	Defines the last sample well
Escape	Defines to the Ready Menu

4. Place a vial with transport solvent (mobile phase) in transport vial position 1 (left).
5. Place a 96-well plate with sample in well A1 on the plate holder.
6. Run the series:
   a. Press **Start/Stop** to start the autosampler.
   b. Press [01] **E** to start execution with series number 1.
   c. Press [01] **E** to end execution after series number 1.
   d. Press START to start the analytical run.

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

Note Press DEFAULT ALL in the Ready Menu (Utilities Menu) to erase all series and methods defined in these examples and to restore all settings to the default values.

Keypad Menu Reference

This chapter describes all the menu options available from the front panel keypad and display of the Micro AS autosampler. Menu options are listed in the order they are displayed.

Contents

Ready Menu

The Ready Menu allows you to access basic hardware and software functions that are necessary for the maintenance and operation of the autosampler. The Ready Menu contains the following soft function keys:

Key	Function
PLATES	Press this key to install or exchange plates. Press EXCHANGE to move the plate to the left; in this position the plate can be replaced without damage to the equipment. Press PLATE HOME to move the plate to operating position again.
WASH	Press this key to start a standard wash procedure. All tubing connected to the syringe valve is filled and rinsed with wash solvent.
SYR END	Press this key to move the syringe to end position to replace the syringe or to simplify filling of wash solvent tubing. A syringe volume of wash solvent is aspirated from the wash solvent bottle and the wash solvent tube is filled. Press SYR HOME to dispense the syringe content to syringe waste and to move the syringe to standard operating position again.

Key	Function
UTILS	Press this key to go to the Utilities Menu. If a method protection code is enabled in the System Menu, the code must be entered to access the Utilities Menu. The menu offers the following options: • COPY Press this key to copy a method. Press the appropriate soft function key (MIX, WASH, INJECTION, TIMEBASE) to specify the method type, and then enter the number of the method to be copied. Then, enter a number to define the destination method. Any existing method stored under that number will be overwritten. • ERASE Press this key to erase a method, template, or user program. If Template and User Program are switched off in the System Menu, the soft function keys for erasing a standard Method (mix, injection, wash, timebase) appear. Note that it is not possible to erase the user program if the protection code for the user program is enabled in the System Menu. • LOG Press this key to access the instrument log. Two options are available from this menu: – EVENTS Press this key to display a log of system-relevant events, such as error messages. Use the NEXT and PREVIOUS keys to scroll through the event list. – COUNT Press this key to display the cumulative count of valve actions, days powered on, syringe movements, and other system information. **Note** After every 50000 syringe actions, the following message is displayed: “Lifetime of syringe may be exceeded. Check for possible leakage.” It is recommended that you replace the syringe at this time. If you do not replace the syringe and choose “Do not display this message again,” the message is not be displayed until 50000 more syringe actions have been counted. After every 200000 syringe valve actions, the following message is displayed: “Lifetime of syringe valve may be exceeded. Check for possible leakage.” Contact your local Thermo Fisher Scientific service representative to have the valve replaced and the counter reset. • DEFAULT ALL Press this key to restore all software settings to the default values. All series, methods, templates, and the user program will be erased, unless protected by a protection code. **Note** After restoring settings to default, enter the System Menu and ensure that the System settings are compatible with your hardware configuration.

Key	Function
COOL	Press this key to enter the programming mode for Peltier plate cooling. The programmable temperature range is 4 °C to 40 °C. The maximum cooling capacity is approximately 12 °C below ambient temperature. (Refer to Appendix E, "Specifications," for specifications.) Press ON to switch on cooling, and use the numeric keypad to enter the setpoint value. When the cool option is on the following soft function keys can be selected: • MANUAL Temperature control remains on until switched off again by the user from this menu. • AUTOMATIC Temperature control is switched off after all programmed series have been executed. • DATE-TIME Temperature control is switched on or off (depending on initial state) at a programmed date and time.
SERIAL	Press this key to put the Micro AS in serial mode to allow for control of the autosampler by way of PC (RS232 interface). Select a device identifier in the System Menu (refer to "System Menu" on page 117). If a method protection code was defined in the system settings, the code must be entered to access serial mode. The following soft function keys appear: • PANIC Press this key to begin a stop sequence in which all tubing is rinsed and the valve and I/O ports are reset. At the end of the sequence, serial mode is resumed. • EXIT Press this key to end serial mode and return to the Ready Menu.
SERVICE	For service to the apparatus. To be used by authorized personnel only. The Service Menu is protected by a service code.

System Menu

The System Menu allows you to define and store values for configurable hardware parameters, such as sample loop volume, needle tubing volume, syringe volume, I/O parameters, and so on. Whenever you change the sample loop, needle, or syringe on your autosampler, verify that the System Menu contains the correct settings.

The System Menu also allows you to enable or disable a number of optional software features, such as the use of mix methods, timebase methods, and programmable user templates.

The System Menu settings determine whether certain options appear or do not appear in other menus of the autosampler software. For this reason, you should program the correct settings for your application in the System Menu before going on to the other menus.

When you enter the System Menu, choose one of the following soft function keys:

- MICRO

  Press this key if the autosampler hardware is configured for operation in Micro mode. This is the default hardware configuration for the Micro AS and supports use of the 25 µL syringe and the 10 µL or 20 µL sample loops.

- CONVENTIONAL

  Press this key if the autosampler hardware is configured for operation in Conventional mode. Conventional mode supports the use of larger syringes and sample loops than those supported in Micro mode.

  Note Switching the autosampler from one mode of operation to the other requires that the System Menu settings be reset to their default values. If you attempt to switch the mode of operation, you will see a warning message, "ALL DATA DEFAULT!! PRESS E TO ACCEPT." Press **E** to switch modes and reset all System Menu settings. Press **CL** to cancel the change and leave System Menu settings as they are.

After you have chosen the mode of operation, you can program the system settings using the following soft function keys:

- GENERAL

  Press this key to enter values for:

  - **loop volume, needle tubing, syringe volume**: Enter these values to reflect the installed sample loop, needle, and syringe. Note that when the autosampler is operated in Micro mode, the syringe volume is automatically set to 25 µL and cannot be varied.

  - **syringe speed and scale factor**: The aspirating speed of the syringe used in injection methods can be set to either low, normal, or high to accommodate differing sample viscosities. In addition, for finer control of the syringe speed, you can enter a scaling factor between 0.1 and 1.0. The specified syringe speed is reduced by multiplying by the scaling factor. The speed of the syringe during the wash or the rinsing procedure of the buffer is not affected by this setting.

  - **needle height:** This parameter defines the distance between the needle point and the plate holder. (The default distance is 5 mm.) The value is only used in injection methods; for mix methods this value is programmable in the method itself.

  - **skip missing vials**: This parameter appears only if a plate with 48 vials is selected in System Menu (Plates Menu). YES means that empty spaces are skipped during the run. NO means that the Micro AS stops if a missing vial is encountered during the run; an error code is generated.

  - **air segment**: This parameter allows you to choose whether an air segment will be used for analytical runs. For more information about the air segment, refer to "Injection Methods" on page 9.

- **headspace pressure**: This parameter allows you to switch headspace pressure on or off. The Micro AS uses headspace pressure to facilitate transport of sample into the loop. Headspace pressure will always be used during a wash procedure. Note that accuracy and reproducibility might decrease if headspace pressure is switched off. However, headspace pressure is only useful if sample wells are airtight. Refer to "Replacing the Needle Assembly" on page 48 and "Replacing a Sample Plate" on page 50 for more information.
- **time display**: This parameter offers a choice between two types of time representation. Select HH:MM:SS to display the time in hours, minutes, and seconds. Select HH:MM.mm to display the time in hours, minutes, and decimal fractions of minutes.
- **key click, error beep and alarm buzzer**: These parameters allow you to switch sound signals on or off.

Table 36 gives an overview of the allowed ranges for the General Menu settings when the autosampler is operated in Micro mode:

Table 36. Ranges for General Menu settings in Micro mode

Parameter	Default	Programmable range
Loop volume	100 µL	0.01 to 25 µL
Needle tubing	15 µL	0.1 to 99.9 µL
Syringe speed	normal	low, normal or high
Scale factor	1.0	0.1 to 1.0
Needle height	5 mm	0 to 40 mm
Skip missing vials	yes	yes or no
Air segment	yes	yes or no
Headspace pressure	no	yes or no
Time display	HH:MM:SS	H:MM:SS or H:MM.mm
Key click	on	on or off
Error beep	on	on or off
Alarm buzzer	ON	ON OR OFF

- USAGE

  Press this key to enter the following Usage settings:

  – **protection code**: Enter a six digit code (000000-999999) for protection of all methods. Press **CL** to erase the code. If a code has been defined it is not possible to enter the System Menu or the Methods Menu without entering the protection code. By default, there is not protection code.

CAUTION If you enter a protection code, be sure to write down the code and keep it in a safe place. Without this code, you will not be able to make changes to the System Menu settings or to programmed methods.

  – **timebase methods**: This parameter allows you to enable the option to program timebase methods. When a timebase method is run, the Micro AS autosampler controls other connected equipment during the analysis time. Program timebase methods in the Methods Menu. By default, timebase methods are not enabled.

  – **mix methods**: This parameter allows you to enable the option to program mix methods for the Micro AS. Program mix methods in the Methods Menu. Note that the Micro AS cannot analyze priority samples during a run if the mix method is enabled. By default, mix methods are not enabled.

  – **user program**: This parameter allows you to enable the option to program a user program. If this function is enabled you can enter a user program protection code (6 digits) that protects the user program from unauthorized editing. (Note that this is a separate protection code from the methods protection code described above. The user program protection code protects the user program only and does not prevent access to the System or Methods Menus.) Program the user program in the Methods Menu. Note that the Micro AS cannot analyze priority samples during a run if the user program is enabled. By default, the user program is not enabled.

  – **labeled wells**: This parameter allows you to enable the option to program labeled wells. When the autosampler reaches a labeled well during a run, it sends an output signal to the P2 TTL marker and P4 contact closure outputs. (Refer to Chapter 3, "I/O Connections," for more information.) These output signals can be used to trigger an external device. Program the location of labeled wells in the Series Menu. By default, the use of labeled wells is not enabled.

  – **templates**: This parameter allows you to enable the option to program templates. Program templates in the Methods Menu. By default, templates are not enabled.

  – **calibration wells**: This parameter allows you to enable the option to program the use of a calibration vial. Program the calibration vial in the Series Menu. By default, calibration wells are not enabled.

Note Switching off all unnecessary options in the Usage Menu ensures that other menus are not cluttered with options that are irrelevant to the types of analyses you want to perform.

- PLATES

  Press this key to define the type of plate to be used. Four types can be selected: 96-low (default), 96-high, 384-low or 48-vials. After a plate type has been selected, enter the following:

  – **well processing method**: Choose processing in rows (left to right) or in columns (top to bottom).

  – **first transport vial**: Enter a number 1 to 4, or press **CL** if transport vials are not used.

  – **last transport vial**: enter a number 1 to 4.

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

- IO

  Press this key to enter the I/O configuration mode and define the following:

  – **inject-marker pulse length**: the length of the inject-marker pulse.

  – **well-marker pulse length**: the length of the well-marker pulse.

  – **labeled well-marker pulse length**: the length of the well-marker pulse of the labeled well.

  – **input edge next injection**: the edge sensitive inputs for the next injection.

  – **input edge next well**: the edge sensitive inputs for the next well.

  – **freeze input active**: whether the freeze input is active when high, or freeze input is active when low.

  – **reset outputs after last series**: whether the outputs should be reset to default after the last series.

  Table summarizes the possible ranges for the I/O parameters. Refer to Chapter 3, "I/O Connections," for more specific information on I/O connections.

Table 37. Summary of allowed values for I/O parameters

Parameter	Default	Range
inject-marker pulse length	1.0 s	0.1 to 2.0 s
well-marker pulse length	1.0 s	0.1 to 2.0 s
labeled well marker pulse length	1.0 s	0.1 to 2.0 s
input edge next injection	falling	falling or rising
input edge next well	falling	falling or rising
freeze input active	low	low or high
reset outputs after last series	no	yes or no

- CLOCK

  Press this key to switch the system clock on or off. Select ON to enter the clock menu in which you can set date (yy,mm,dd) and time (hh,mm). This date and time will be displayed in the Ready Menu.

- COMM

  Press this key to define a device identifier for communication with other equipment (for example, a data system computer). An identifier between 20 and 29 can be selected for the Micro AS.

Methods Menu

This menu allows you to program various types of methods. It is possible to define 24 separate injection methods, 5 wash methods, 5 timebase methods, 9 mix methods, and one user program.

It is also possible to program a combination of methods and save them in a **template**. The settings entered in the System Menu determine the options offered by the Methods Menu.

- TEMPLATE

  Press this key to enter a menu in which a template can be defined. First assign a number to the template, then link the numbers of methods to the template. The following items can be entered to fill a template:

  – **user program instead of methods**:

    - If soft function key YES is selected, the complete template is filled with the user program; no other methods can be added.
    - If soft function key NO is selected, the following can be entered into the template: mix method number, injection method number, wash method number, and timebase method number.

  You can program a maximum of 24 templates.

- METHODS

  Press this key to enter a menu for defining the following types of methods:

  – MIX (if enabled in System Menu, Usage Menu)

    Press this key to program a method that allows you to perform pre-injection sample handling, such as pre-column derivatization, dilution or adding of internal standard. Nine mix methods can be programmed; the maximum number of steps that can be programmed for the total of 9 mix methods and the user program is 240. After you assign a number to the mix method, the Mix Menu appears with the following soft function keys:

    - EDIT: An existing step or a new step for a new mix method.
    - INSERT: A new step in an existing method before the displayed step.
    - DELETE: The displayed step.

    If the mix method is empty, “End of mix method” is displayed. If an existing mix method is selected, the first line of the mix method is displayed. Scroll through the steps of the existing method with the cursor keys and use the soft function keys to enter changes in an existing method.

    The following types of steps can be programmed for a mix method:

    - ASPIRATE: Aspirate a programmed volume of sample, air, liquid from destination vial, or liquid from reagent vial. The syringe speed can be set to a value between 1 and 9. The height (H) indicated is the distance from the needle point to the plate holder. (The default height is 5 mm.) The maximum aspiration volume is equal to the total volume of the syringe.
    - DISPENSE: Dispense a programmed volume from the buffer tubing into a sample, destination, or reagent vial, or to waste. The syringe speed can be set to a value between 1 and 9. The height (H) indicated is the distance from the needle point to the plate holder. (The default height is 5 mm.) It is possible to dispense a larger volume than that aspirated in previous actions. In that case, the aspirated amount is complemented with liquid from the wash solvent bottle to equal the total programmed dispense volume.

CAUTION During a dispense action, the pressure in the buffer tubing increases. To prevent damage to the buffer tubing, do not allow flow rates to exceed 6 mL/min for water or liquids of similar viscosity. See Table 38 for flow rates achieved by different syringes at different speeds.

- WAIT: Pause for the programmed period of time (H:MM:SS). Maximum wait time is 9 hours, 59 minutes, and 59 seconds.

  Note During the pause, the needle moves to the home position (if the previous step was an aspirate or dispense action). If you want the needle to stay in the same position, program an aspirate or dispense step of 0 µL at the desired position.

- REPEAT: Designate a sequence of previous steps to be repeated, and specify how many times they will be repeated.
- WASH: Enter the volume for a needle wash. Buffer is rinsed to waste.
- INJECTION: Use this key to program an injection method for a run of the type full loop, partial loopfill or µL pick-up. Enter a number for the injection method you are going to program.

  Note If you have programmed changes in the System Menu that are not compatible with a programmed injection method, the method will be locked and you will not be able to use it in a run. If the selected method is locked, the word 'LOCK' is displayed. You can unlock a method by restoring the System Menu settings to their previous values, or by programming new valid parameters in the injection method itself.

  Use the soft function keys to select an injection method, then enter values for:

  – **analysis time**: the time between switching the injection valve to inject and the start of processing the next sample.

  – **flush volume**: the amount of sample taken from a vial before the loop is filled with sample. The default value is 5 µL.

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

  – **number of injections per vial:** maximum value is 9.

  – **injection volume:** can be entered for each injection per vial. For maximum injection volumes, refer to "Injection Methods" on page 9.

  – **valve to load:** Use this option to switch the injection valve back to the LOAD position after injection. Enter a value for the time between start of injection and switching back to LOAD. If the time is set to 00:00:00, the valve will remain in the INJECT position until the next programmed injection sequence, if any.

- WASH

  Use this key to program wash methods. It is possible to program a wash between injections, samples or series. The default wash volume is 300 µL.

- TIMEBASE (if enabled in System Menu, Usage Menu)

  Press this key to enable control of external devices via auxiliary or binary outputs. A maximum of 5 timebase methods can be programmed. The menu offers the following soft function keys:

- AUX

  Scroll through all program lines by pressing **E**, or press AUX to move to the next auxiliary output.

- VALVES

  The Micro AS autosampler does not use this function.

- CODE

  Enter a time and a value between1 and 15, hexadecimal output. Press **E** to scroll through the programming lines.

- END

  Enter the end time for a timed events program. If no value is entered, or if **CL** is pressed, an end time is generated automatically based on the analysis time programmed for the injection method used in the relevant series.

  Note If the programmed end time exceeds the programmed analysis time, the end time overrides the analysis time. It is possible to program events after the end time, but these events are not executed during a run.

- USER PROGRAM

  The user program allows you to program all possible actions required for a sample handling sequence in separate steps. Note that the total number of steps for the user program and all nine mix methods cannot exceed 240. The user program can be protected by a special user program protection code (System Menu, Usage Menu). If no user program has been programmed yet, “end of user program” is displayed. Otherwise, the first line of the programmed method appears. The following soft function keys are available:

  – EDIT: An existing step or program a new step for the user program

  – INSERT: A new step before the displayed step

  – DELETE: Delete the displayed step.

The Edit and Insert Menus offer the following soft function keys:

- ASPIRATE

  Aspirate a programmed volume from a sample well, air, a destination vial, wash, or one of the reagent vials into the buffer tubing. Syringe speed and needle height can be entered. The maximum volume that can be aspirated is the total volume of the syringe.

- DISPENSE

  Dispense a programmed volume from the buffer tubing into a sample well, waste, destination vial, wash, or one of the reagent vials. The syringe speed and needle height can be entered. Unlike DISPENSE actions programmed in a mix method, DISPENSE actions in a user program cannot be set to dispense a larger volume than that aspirated in previous actions.

CAUTION During a dispense action, the pressure in the buffer tubing increases. To prevent damage to the buffer tubing, do not allow flow rates to exceed 6 mL/min for water or liquids of similar viscosity. See Table 38 for flow rates achieved by different syringes at different speeds.

- SYR_VALVE

  Press this key to control the connection between the syringe and other tubing:

  – NEEDLE: Press this key to connect the syringe with the sample needle.

  – WASH: Press this key to connect the syringe with the wash solvent bottle.

  – WASTE: Press this key to connect the syringe with the syringe waste tubing.

- SYR

  Press this key to control the movements of the syringe:

  – LOAD: Press this key to load the programmed volume.

  – UNLOAD: Press this key to unload the programmed volume.

  – HOME: Press this key to dispense a previously aspirated volume to the last programmed position, and then re-initialize syringe.

- WASH

  Press this key to execute a needle wash. The content of the buffer tubing is not rinsed to waste before the start of the wash. The programmed volume of wash solvent is used to wash the needle at the wash position.

Note The wash position might become contaminated with the contents of the buffer tubing, which can lead to cross-contamination. To prevent contamination of the wash position, program a dispense to waste action before programming a wash action.

- VALVES

  Press this key to program positions of high pressure valves. The injection valve has two positions, INJECT and LOAD.

- WAIT

  Press this key to program a pause. The maximum wait time is 9 h, 59 min, and 59 s.

  Note During the pause, the needle moves to the home position (if the previous step is an aspirate or dispense action). If you want the needle to stay in the same position, an aspirate or dispense step of 0 µL must be programmed at the desired position.

- COMPRES

  Press this key to activate the compressor to put air pressure on a sample. The compressor stays active until it is switched off in a subsequent programmed step.

- AUX

  Press this key to control the contact closure auxiliaries (connector P5). Refer to Chapter 3, "I/O Connections," for more information.

- WAIT-IN

  Press this key to program a pause during which the Micro AS waits for one of the four inputs (connector P6) to become HIGH or LOW before continuing with the next step. Refer to Chapter 3, "I/O Connections," for more information.

- PROG-OUT

  Press this key to program the contact closure programmable outputs (connector P1). These outputs are similar to the auxiliaries, but are only available in the user program. Refer to Chapter 3, "I/O Connections," for more information.

- CODE

  Control the timed output (connector P3). This is a hex output in the range 0 to 15. Refer to Chapter 3, "I/O Connections," for more information.

- MARKERS

  The markers normally generated by the Micro AS are not active during a user program by default, but can be activated by choosing this menu option. Select marker and status (inject, vial, or labeled).

- SSV

  This function is not used with the Micro AS autosampler.

When programming dispense actions in mix methods and user programs, it is important to take into account the liquid flow rate. When liquid is dispensed, the pressure in the buffer tubing increases. If the flow rate is too high, the buffer tubing can be damaged. For water, or liquids of similar viscosity, the flow should not exceed 6 mL/min. If more viscous liquids are used, the flow should be reduced.

Table 38 shows flow rates for various combinations of syringes and syringe speeds. The gray shaded area indicates speeds that exceed the recommended maximum flow of 6 mL/min.

Table 38. Flow rates for various syringe speeds and syringe volumes

Speed	Syringe volume					
	25 µL	100 µL	250 µL	500 µL	1000 µL	10 µL
1	12.5 µL/min	50 µL/min	125 µL/min	250 µL/min	500 µL/min	0.5 mL/min
2 (low)	31 µL/min	125 µL/min	315 µL/min	630 µL/min	1255 µL/min	1.3 mL/min
3 (normal)	63 µL/min	250 µL/min	625 µL/min	1250 µL/min	2495 µL/min	2.5 mL/min
4 (high)	94 µL/min	375 µL/min	940 µL/min	1880 µL/min	3765 µL/min	3.8 mL/min
5	193 µL/min	770 µL/min	1920 µL/min	3840 µL/min	7680 µL/min	7.7 mL/min
6	268 µL/min	1070 µL/min	2675 µL/min	5335 µL/min	10665 µL/min	10.7 mL/min
7	343 µL/min	1370 µL/min	3430 µL/min	6855 µL/min	13710 µL/min	13.7 mL/min
8	436 µL/min	1745 µL/min	4365 µL/min	8725 µL/min	17450 µL/min	17.5 mL/min
9	534 µL/min	2135 µL/min	5335 µL/min	10670 µL/min	21335 µL/min	21.3 mL/min

Series Menu

This menu allows you to define the run sequence in a series. A maximum of 24 series can be programmed. A series contains information about the methods to be used for a range of wells. This can be a template, a separate method (mix, injection, wash, timebase), or the user program. Information on location of wells, labeled wells or calibration wells is also programmed in a series.

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

After you have entered the required settings in the System Menu and after you have programmed methods to be used for an analytical run, press **Series** to enter the Series Menu. Figure 74 gives an overview of the parameters you need to define for each series. Parameters shown in bold in this figure must be set for all series. The availability of other parameters depends on the method used and the settings enabled in the System Menu.

Two types of series can be programmed:

- Series With Templates
- Series Without Templates

Note Series are stored in the Micro AS memory for as long as the power is on. As soon as power is switched off, all programmed series are deleted.

It is not possible to leave the Series Menu until all values have been programmed

Series With Templates

To program a series with a template, enter the template number, the location of the first sample well, and the location of the last sample well. If your System Menu settings have been configured correctly, you can also enter parameters for calibration vials, mix methods, and labeled wells. See Figure 74 for the order in which these parameters are programmed.

If you have enabled the use of calibration vials in the System Menu (Usage Menu), specify whether you will use calibration vials and indicate the number of wells between calibration vials. See Figure 75 for an illustration of an example calibration sequence.

If you have enabled the use of a Mix Method in the System Menu (Usage Menu), specify the location of the first destination well and of the reagent vials.

Series Without Templates

To program a series without a template, enter an injection method number, a wash method number, the location of the first sample well, and the location of the last sample well. If your System Menu settings have been configured correctly, you can also enter parameters for the user program, time base methods, calibration vials, mix methods, and labeled wells. See Figure 74 for the order in which these parameters are programmed.

If you have enabled the use of calibration vials in the System Menu (Usage Menu) specify whether you will use calibration vials and indicate the number of wells between calibration vials. See Figure 75 for an illustration of an example calibration sequence.

If you have enabled use of a Mix Method in the System Menu (Usage Menu), specify the location of the first destination well and of the reagent vials.

Figure 74. Series programming parameters

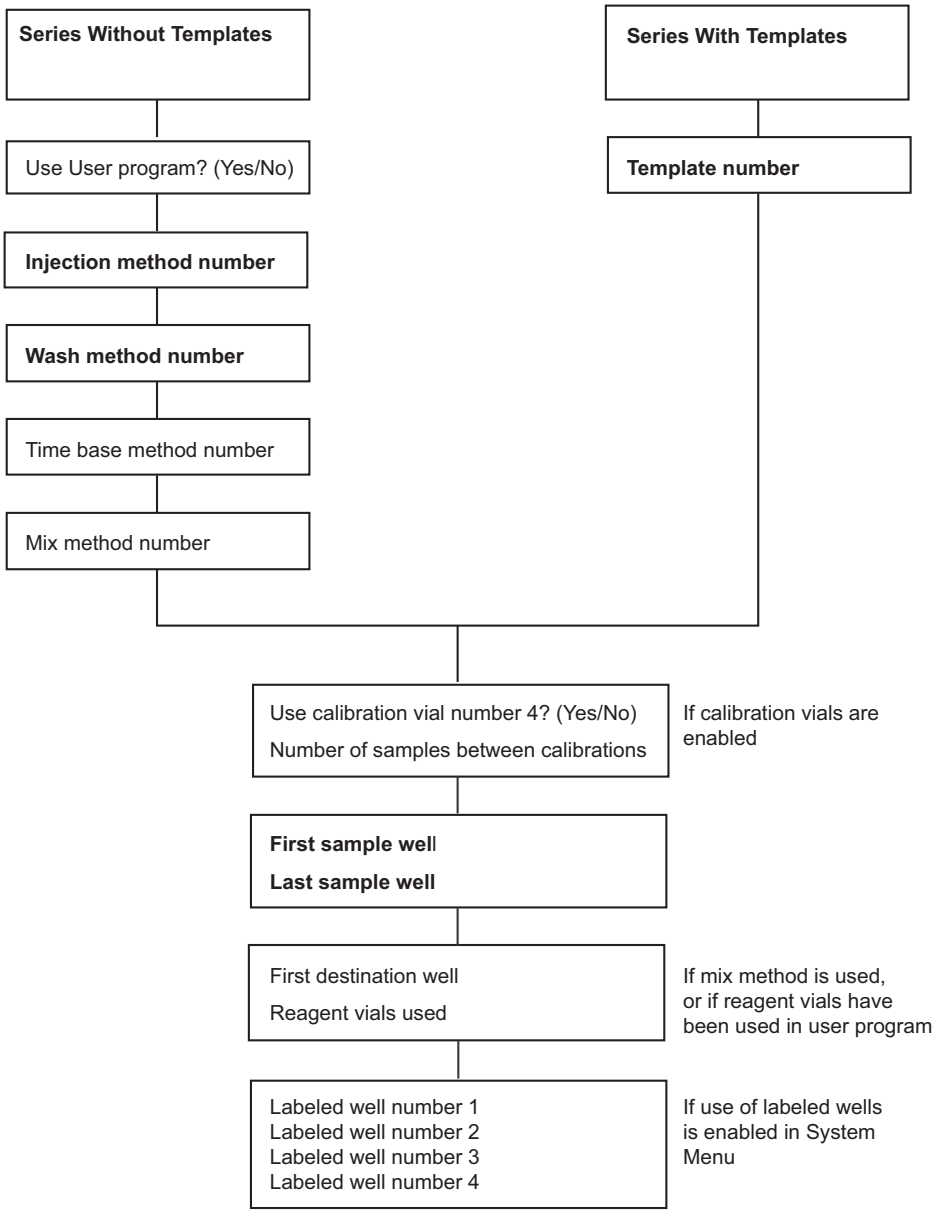
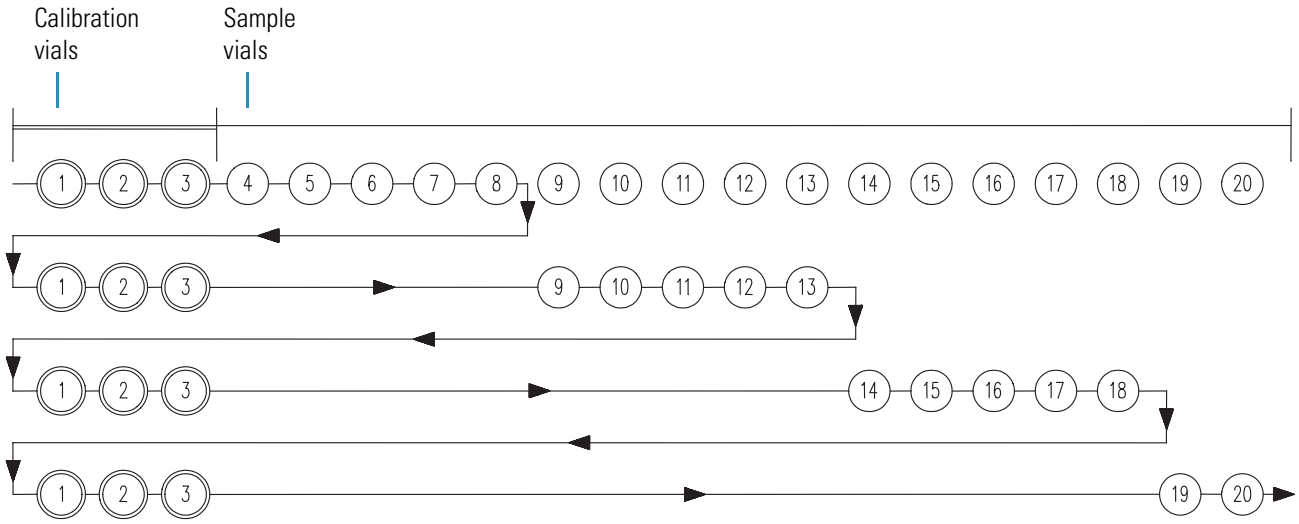


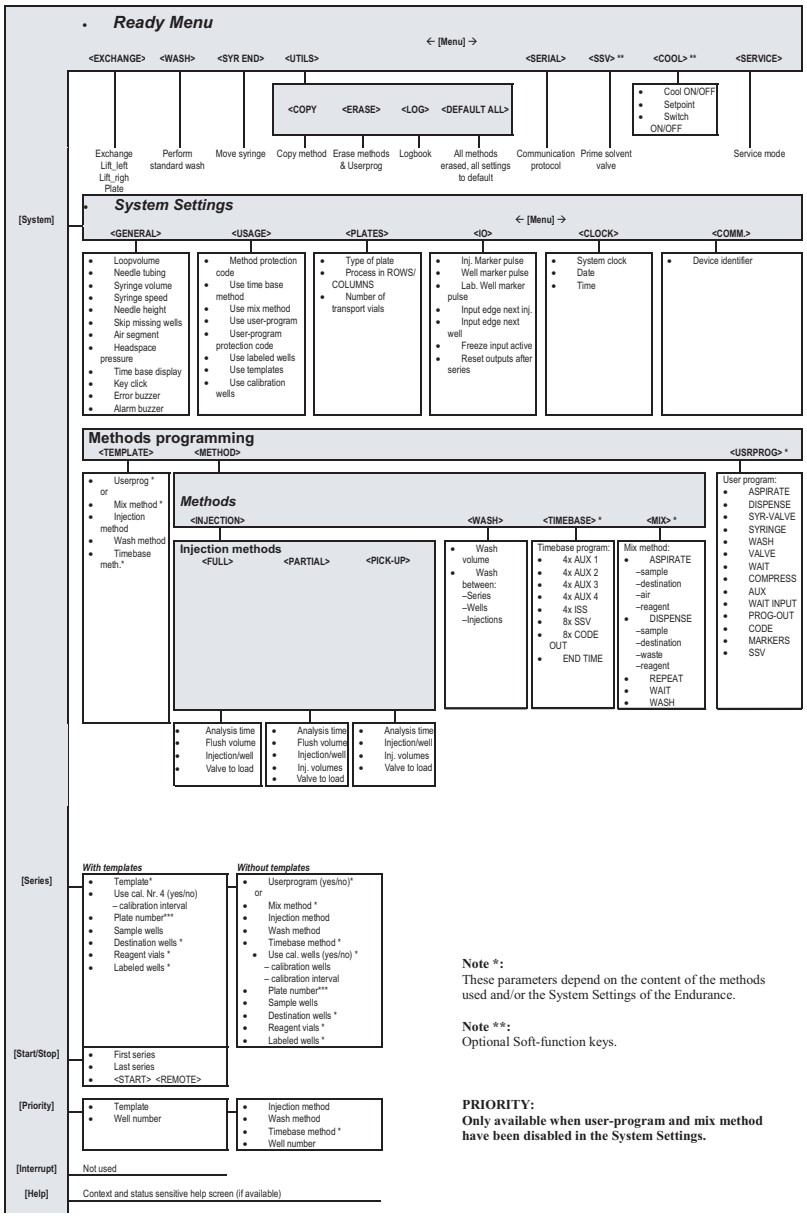
Figure 75. Injection sequence with three calibration vials after every five sample vials



Programming Reference Chart

The chart in Figure 76 shows all of the programming options for the Micro AS autosampler.

Figure 76. Autosampler programming options



Logbooks

This chapter contains sample pages for logbooks that can be used with the Micro AS autosampler. Use photocopies of the pages provided to keep records of system settings and programmed methods and templates for future reference.

Contents

Instrument Information

User Information

Name of user:
Company:
Department:
Address:
Telephone:
Fax

Micro AS Information

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

System Menu Settings

Micro _____ or Conventional _____					
General			**Usage**		
Loop volume		_____ µL	Protection code		_____
Needle tubing volume		_____ µL	Timebase methods		____ enabled ____ off
Syringe volume		_____ µL	Mix methods		____ enabled ____ off
Syringe speed	_____ µL	factor: ____	User program		____ enabled ____ off
Needle height		_____ mm	User program protection code		_____
Skip missing vials	____ yes	____ no			
Air segment	____ yes	____ no	Labeled wells		____ enabled ____ off
Headspace pressure	____ yes	____ no			
Time display	HH:MM:SS	HH:MM:mm	Templates		____ enabled ____ off
Key click	____ on	____ off			
Error beep	____ on	____ off	Calibration wells		____ enabled ____ off
Alarm buzzer	____ on	____ off			

Plates	**IO**		
____ 96-low	Inject-marker pulse length		____ s
____ 96-high	Well-marker pulse length		____ s
____ 384-low	Labeled well-marker pulse length		____ s
____ 48-vials	Input edge next injection	____ falling	____ rising
Processing in: ____ Rows ____ Columns	Input edge next well	____ falling	____ rising
Position first transport vial ____	Freeze input active	____ low	____ high
Position last transport vial ____	Reset outputs after last series	____ yes	____ no

Clock	**COMM**
____ on ____ off	Device indentifier: 2 ____

Comments:

Templates

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

Comments:

Injection Methods

Method #	Injection type	Analysis time	Flush volume	Injection per well	Valve to load	Injection volumes: 1	2	3	4	5	6	7	8	9
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
15														
16														
17														
18														
19														
20														
21														
22														
23														
24														

Comments:

Wash Methods

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

Timebase Methods

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

Mix Methods

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

Comments:

User Program

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

Comments:

Specifications

This chapter describes the specifications for the parts and operation of the Micro AS. The following types of specifications are given:

- General Specifications
- Analytical Performance Specifications
- Programming Specifications
- Physical and Electrical Specifications
- Communication Specifications

General Specifications

Table 39 gives the general specifications for the Micro AS autosampler.

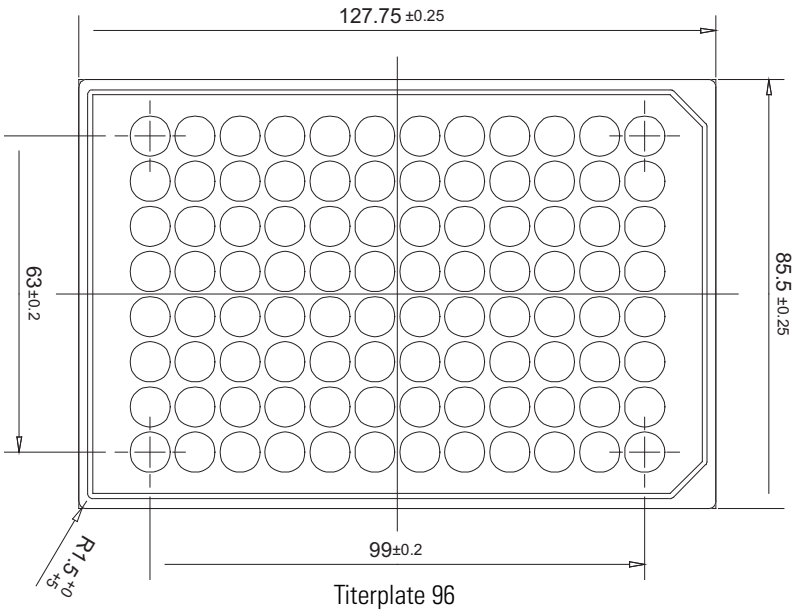
Table 39. Micro AS general specifications

Parameter	Specification
Sound pressure level	Less than 70 dB (Leaq)
Working temperature	5 to 40 °C
Storage temperature	-25 to +60 °C
Humidity	20% to 80% relative humidity
Sample viscosity	0.1 to 5 cP
Plates (refer to Figure 77)	Types: 48 vials, 96 low, 96 high, 384 low Maximum height vials: 47 mm (incl. cap) Minimum height vials: 12.5 mm (incl. cap). Only plates of rigid material can be used.
Vial dimensions	6 × 8 vials, dimensions base plate 128 × 85.8 mm Maximum outer diameter for 48-vials: 12 mm
Reagent/transport vials	Dimensions 10 mL vials: 22 × 47 mm (maximum height including cap)
Loop volume	5 to 20 µL
Puncturing needle accuracy	± 0.5 mm
Pre-puncturing septa/caps	With air needle, dual needle action
Plate & height detection	Missing plate & height detection by vial sensor
Plate tray drive	Greater than 5 cm/s
Sample cooling	Built-in Peltier cooling processing unit Programmable Range : 4 °C to 40 °C Cooling capacity: Ambient -12 °C (measured on cooling plate)
Injection valve switching time	(Electrically) Less than 100 ms
Headspace pressure	Built-in compressor
Wash solvent	250 mL internal wash solvent bottle
Dispenser syringe	25 µL syringe in Micro mode.
Wetted parts	SS316, PTFE, Tefzel™, Vespel™ Glass, Teflon™, PEEK™, Fused silica

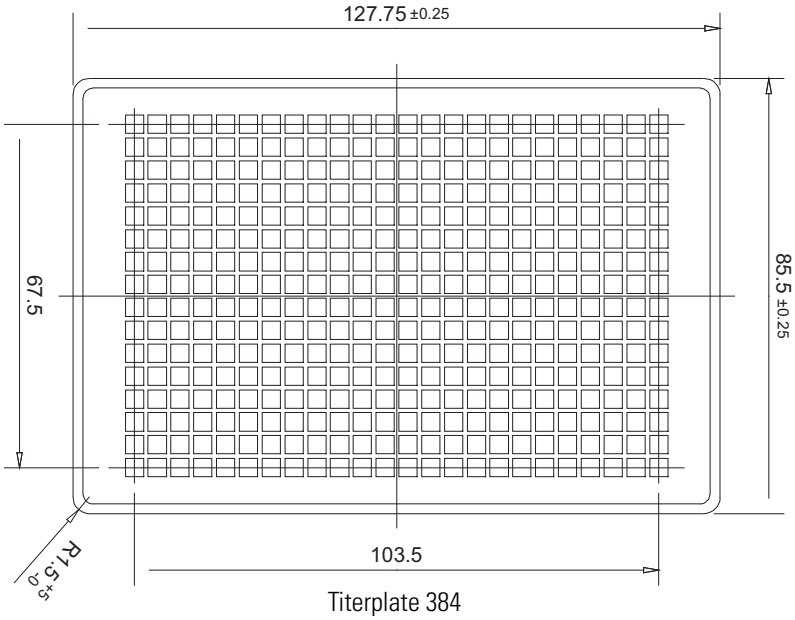
Figure 77 illustrates the specified dimensions of compatible titerplates. The sides and ends of plates will be straight within ±0.50 mm. This is defined as the maximum variation from a theoretical line connecting the applicable outside corners. When the plate is measured at its point of maximum deflection, dimensions will be as follows:

- Length:127.75 ± 0.50 mm (384 titerplate and 96 titerplate)
- Width: 85.5 ± 0.50 mm

Figure 77. Schematic of titerplates, showing dimensions of compatible plates



Titerplate 96



Titerplate 384

Analytical Performance Specifications

Table 40 gives the analytical performance specifications of the Micro AS as measured using capped and sealed vials.

Table 40. Analytical performance specifications

Parameter	Specification
Full loop injections	RSD ≤ 0.3%
Partial loopfill injections	RSD ≤ 1.0%, injection volumes greater than 0.2 µL, with headspace pressure on the vial and 6 µL pre-flush without air segment.
µL pick-up injections	RSD ≤ 1.0 %, injection volumes greater than 0.2 µL, with headspace pressure on the vial.
Memory effect	< 0.01% with programmable needle wash

Programming Specifications

Table 41 gives the programming specifications for the Micro AS.

Table 41. Programming specifications

Parameter	Specification
Injection methods	Full loop, partial loopfill, and µL pick-up injections
Injection volume	Full loop: 0.01 to 99 µL Partial loopfill: 0.01 to 99 µL in 0.01 µL increments µL pickup: 0.01 µL - max. volume in 0.01 µL increments. Max. volume = (loop volume - 3 × needle volume)/2
Injections per vial	Maximum of 9 (volumes are programmable for each injection)
Analysis time	Maximum of 9 h 59 m 59 s
Needle wash	Programmable (between injections, wells, or series)
Priority sample	Freely programmable
Series	Freely programmable

Physical and Electrical Specifications

Table 42 gives the physical and electrical specifications for the Micro AS.

Table 42. Physical and electrical specifications

Parameter	Specification
Dimensions	280 mm × 400 mm × 440 mm (11 in. × 15.75 in. × 17.3 in.)
Weight	26 kg (57.2 lbs.)
Power requirements	115 V ac; +15/-20%; 50 Hz/60 Hz; 250 VA 230 V ac; +15/-20%; 50 Hz/60 Hz; 250 VA
Fuses	For 115 V ac: two 5.0 AT-fuses (¼ in. × 1¼ in., UL/CSA) For 230 V ac: two 2.5 AT-fuses (5 × 20 mm, IEC 127) All fuses UL-listed and CSA-certified

Communication Specifications

Table 43 gives the communications specifications for the Micro AS.

Table 43. Communications specifications (Sheet 1 of 2)

Parameter	Specification
OUTPUTS	Inject marker
	Well marker
	Labeled well marker
	Stop I/O
	4 Auxiliary outputs
	2 Programmable outputs
	Alarm output
	4-bit timebase
INPUTS	Next injection input
	Next well input
	Freeze input
	Stop I/O
	4 Programmable inputs

Table 43. Communications specifications (Sheet 2 of 2)

Parameter	Specification
Serial Communication Port	RS232C or RS422 or RS485
PC-control	• Microsoft Windows® XP • Xcalibur 2.0 with LC Devices 2.0.2

1User Programs

This chapter contains a few examples of the user programs you can create to perform special functions with the Micro AS autosampler.

Contents

Programmable Actions

You can add the following actions to a user program:

- Aspirate
- Auxiliary (P5)
- Code Output (P3)
- Compressor
- Dispense
- Marker (P4)
- Output (P1)
- Syringe Valve
- Syringe
- Valve
- Wait
- Wait for Input (P6)
- Wash

Action	Description
Aspirate	Pulls sample, air, or reagents into the syringe. You can specify up to 4 different reagent vials (Reagent A, Reagent B, Reagent C, or Reagent D) or a sample well (Sample or Destination well), how much to aspirate up to the syringe volume, how fast this is done, and how deep (mm from bottom) the needle dips into the liquid container. The Sample and Destination wells are designated in the Xcalibur sequence set up. If Wash is selected, the designated volume is pulled into the needle from Reagent A vial and then dispensed to waste.
Auxiliary (P5)	Controls the contact closure at connector P5. Use this action to send a start signal to the MS detector and the optional sample pump.
Code Output (P3)	Programs output to connector P3.
Compressor	Turns the compressor ON or OFF. The compressor creates headspace pressure in the sample well to prevent bubble formation during aspiration.
Dispense	Injects a specified amount from the syringe into either a sample or destination well, a reagent vial, or to waste.
Marker (P4)	Generates markers normally generated by the autosampler in other modes at connector P4. Use this action to signal the MS pump that the autosampler is ready to inject.
Output (P1)	Programs output contact closures at pin 1 or 2 of connector P1.
Syringe Valve	Controls the position of the three-port syringe valve. The valve can switch the connection of the syringe to the needle, the waste tubing, or the wash bottle.
Syringe	Specifies the movement of the syringe: • HOME. The volume previously aspirated is dispensed to the last programmed position. The syringe is then initialized. • LOAD. Loads the syringe with the specified volume. • UNLOAD. Unloads the specified volume from the syringe.
Valve	Switches the high pressure valve to the inject position or the load position.

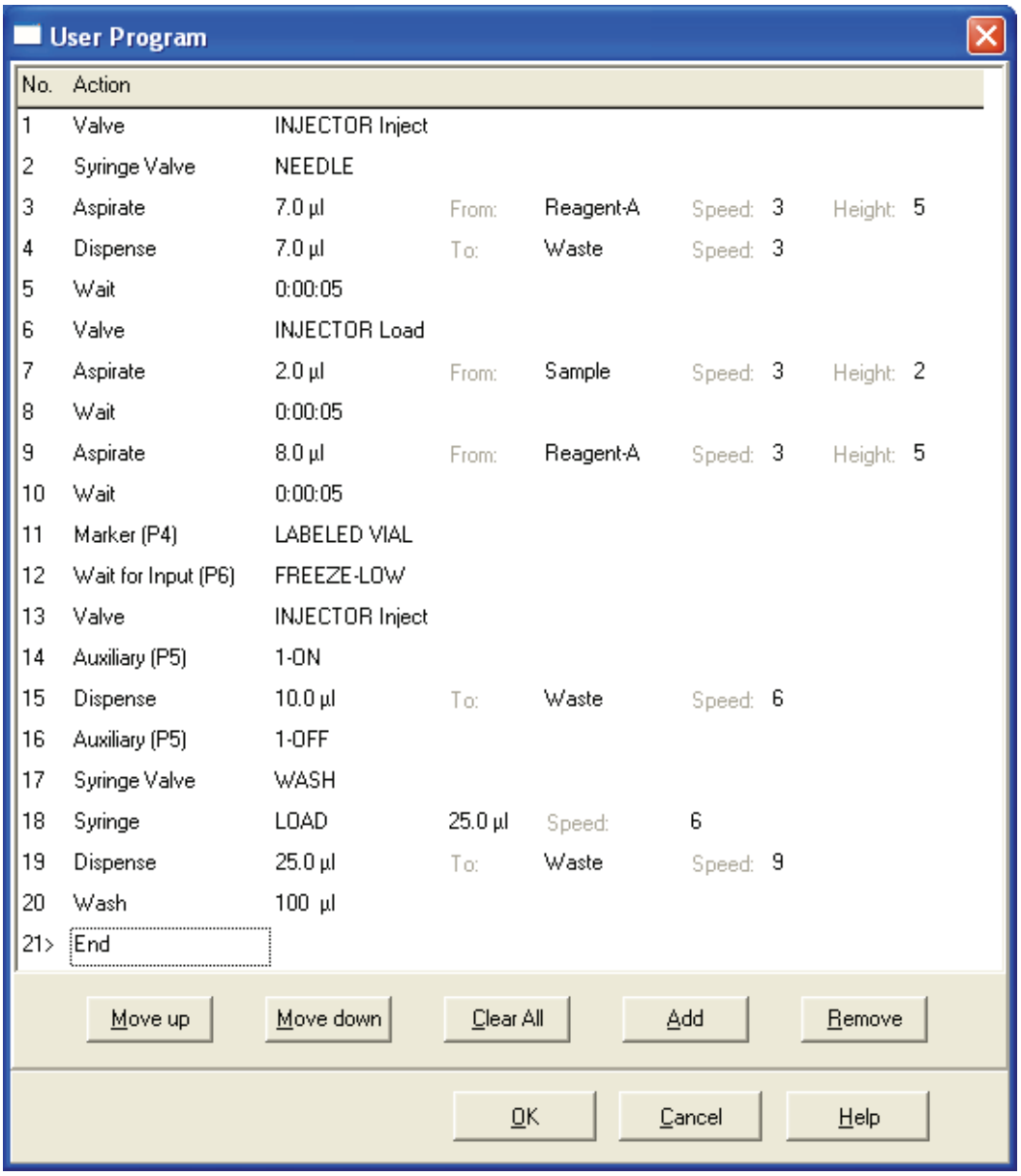
Action	Description
Wait	Creates a waiting period between events. You must specify the hours:minutes:seconds of the waiting period.
Wait for Input (P6)	Pauses the program for an input signal (Freeze Input) to connector P6 and specifies whether to look for a high or low signal. Use this action to synchronize the home position of the pump cam with the switching of the injection valve to the inject position so that the sample is always injected at the beginning of the gradient program.
Wash	Washes the needle with a specified volume of wash solution that is expelled to waste.

Simulated µL Pickup Injection

Figure 78 shows a simulated µL Pickup injection. The standard µL Pickup injection is described in "µL Pick-up" on page 18.

When you create a user program, it is important to set an appropriate speed for aspirate or dispense actions. Decreasing the aspirate or dispense rate increases the precision of the transfer volume. It is also important to add a waiting period of at least 5 seconds after an aspirate or dispense action that is being used to quantitatively transfer fluid.

Figure 78. Simulated µL Pickup injection method



The following table lists the compares the steps of this user program to the standard µL Pickup injection method.

Step	Standard	Simulated
1	Injection valve is switched to the Load position.	same
2	Syringe valve is switched to the Needle position.	same
3	Transport liquid is drawn from the transport liquid vial.	8 µL of Reagent A is aspirated into the needle.
4	Transport liquid is expelled into the loop.	8 µL of Reagent A is expelled from the needle. The reagent is pushed through the injection valve to waste.
5		5 second waiting period
6	Injection valve is switched to the Load position	same
7	The injection volume specified in the main page of the program is aspirated into the needle.	2 µL of sample is aspirated into the needle
8		5 second waiting period
9	The volume of transport liquid aspirated into the needle depends on the XXX. The	8 µL of Reagent A is aspirated into the needle.
10		5 second waiting period
11	The autosampler sends a Ready output signal to the MS pump.	same
12	The autosampler waits for a Pump ready signal from the MS pump. The MS pump sends this signal when its cams reach the home position.	same
13	Injection valve is switched to the Inject position	same
14	The autosampler sends a start signal to the MS detector and optional sample pump.	same
15		Pushes 10 µL of XXX through the injection valve to waste.
16	The autosampler turns off the start signal.	
17	The syringe valve switches to the wash position.	
18		The syringe draws 25 µL of wash solvent into the needle.
19		The needle dispenses the wash solvent through the injection valve to waste.
20		Washes the needle with 100 µL of wash solution.

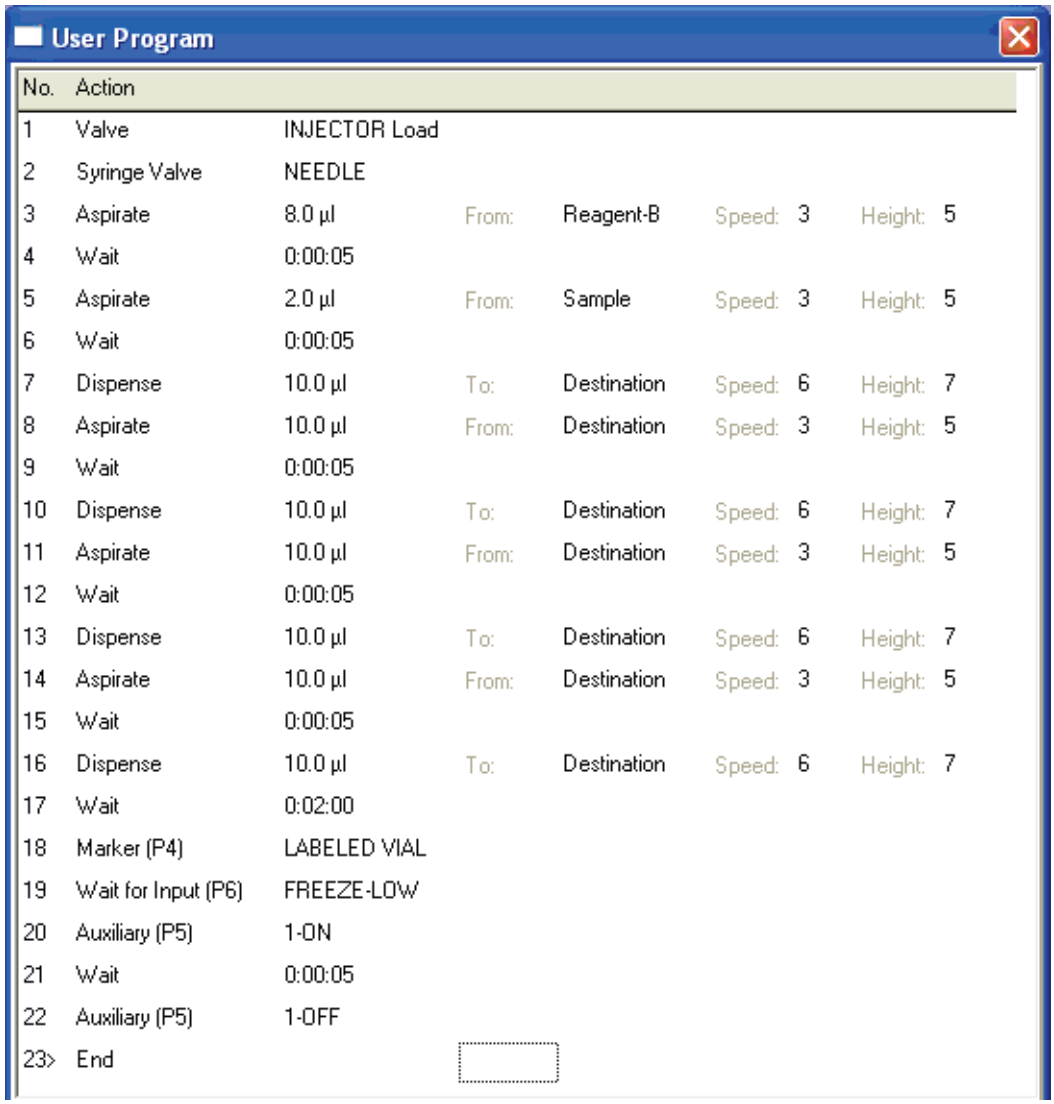
Sample Dilution User Program

Figure 79 shows an example of a sample preparation method in which the sample is diluted 5-fold. The program steps are describe below.

Step	Action
1	Switch the injection valve to the load position.
2	Switch the syringe valve to the needle position.
3	Aspirate 8 µL from the reagent B vial (diluant) at a speed of 3 µL/s and from a height of 5 mm.
4	Wait for 5 seconds.
5	Aspirate 2 µL from the sample location (vial specified in the sequence) at a speed of 3 µL/s and from a height of 5 mm.
6	Wait for 5 seconds.
7	dispenses the sample and diluant into a destination location
	Steps 8 through 16 mix the solution in the destination location three times.
8	Aspirate 10 µL from the destination location at a speed of 3 µL/s and from a height of 5 mm.
9	Wait for 5 seconds.
10	Dispense 10 µL into the destination location at a speed of 6 µL/s and to a height of 7 mm.
11	Aspirate 10 µL from the destination location at a speed of 3 µL/s and from a height of 5 mm.
12	Wait for 5 seconds.
13	Dispense 10 µL into the destination location at a speed of 6 µL/s and to a height of 7 mm.
14	Aspirate 10 µL from the destination location at a speed of 3 µL/s and from a height of 5 mm.
15	Wait for 5 seconds.
16	Dispense 10 µL into the destination location at a speed of 6 µL/s and to a height of 7 mm.
17	Wait for 2 minutes. This step allows the mixed solution to equilibrate.

Step	Action
	Steps 18 through 22 prepare the MS detector and the MS pump for a subsequent injection of the diluted sample.
18	Send a Ready for Inject signal to the MS pump.
19	Wait for a signal from the MS pump that its cams have reached the home position.
20	Send a start signal to the MS detector and optional sample pump.
21	Wait for 5 seconds.
22	Turn off the start signal.
23	End of user program

Figure 79. User program, showing an example in which a sample is diluted 5-fold



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