

# **UltiMate 3000 Nano and Cap**

# **System Installation and Application**



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## **1** Introduction

#### 1.1 General

When working with analytical instrumentation, you should know the potential hazards of using chemical solvents.

This document is intended as a supplementary guide to the *Operating Instructions* for the individual modules of an UltiMate 3000 system (also called "the system" throughout the manual), providing information about the installation and application of the entire system for nano and capillary HPLC. The descriptions in the manual apply to the standard (stainless steel) and biocompatible versions of the system modules. If some detail applies to only one version, the version is identified by name.

In order to obtain a full understanding of the system, Dionex recommends that you review this document and the *Operating Instructions* for the system modules thoroughly before beginning installation and operation of the system.

At various points throughout the document, messages of particular importance are indicated by certain symbols:

1	Please note:	Indicates general information intended to optimize the performance of the instrument.			
Δ	Important:	Indicates that failure to take note of the accompanying information may result in damage to the instrument.			
Δ	<b>M</b> Important: Indique que ne pas tenir compte de l'information jointe endommager l'instrument.				
STOP	Warning:	Indicates that failure to take note of the accompanying information may result in personal injury.			
STOP	Avertissement: Indique que ne pas tenir compte de l'information jointe peut entraîne des blessures corporelles.				

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<sup>1</sup> 

#### 1.2 Safety Precautions

To avoid the possibility of personal injury and/or damage to the UltiMate system, observe the safety precautions for the individual system modules ( $\rightarrow Operating Instructions$  for the instruments).

- **M** Important: When connecting the capillaries, make sure that the connectors are free from contaminants. Even minute particles may cause damage to the flow splitter, flow control valve, and column.
- ▲ Important: Lorsque vous connectez les capillaires, assurez-vous que les raccords sont exempts de tout contaminant. Même d'infimes particules peuvent causer des dommages au système (ex. diviseur de débit, vanne de régulation de débit et colonne).
- **1** Please note: Use only solvents and bottled water of at least HPLC Grade (0.2 μm, filtered), or better LC-MS Grade, and buffers that are compatible with components in the flow path of the flow manager. If water from water purification systems that are not properly maintained is used, polymeric contamination may seriously damage the column and may rapidly block solvent filters. Note the special properties of the solvents such as viscosity, boiling point, UV absorption (UV/VIS detector), and refractive index (refractive index detector).

Buffer concentration: Typically up to 1 mol/L.

#### 1.3 Unpacking

All electrical and mechanical components of the system modules are carefully tested before the instruments are shipped from the factory. After unpacking, please inspect the modules for any signs of mechanical damage that may have occurred during transit.

- Please note: Immediately report any shipping damage to both the incoming carrier and Dionex. Shipping insurance will compensate for the damage only if reported immediately.
- **Please note:** Keep the original shipping container and the packing material. They provide excellent protection for the instrument in case of future transit. Shipping the instrument in any other packaging automatically voids the product warranty.

Unpack the instruments as follows:

- Place the shipping container on the floor and remove the accessories kit and the power cord.
- Grasp the instrument by the sides. Slowly and carefully, pull unit out of the shipping container and place it on a stable surface.

**△** Important: To prevent the instrument from falling, always lift the unit by the sides. Do not lift the unit by the packaging material or the front panel.

▲ Important: Afin d'empêcher l'instrument de tomber, saisissez-la par les côtés. Ne soulevez l'instrument à l'aide du matériau d'emballage ou par la porte du panneau avant.

- Remove the foam inserts.
- Check off the contents of the accessories kit pack against the accessories list.

**Important:** To prevent damage to the instrument when lifting or moving, always hold it by the bottom or sides.

▲ Important: Afin d'éviter d'endommager l'instrument lorsque que vous le soulevez ou le déplacez, saisissez-la toujours par le bas ou les côtés de l'unité.

#### **1.4 Supported System Configurations**

For an overview of the currently available system modules, refer to the table below. If you have any questions, do not hesitate to contact your Dionex sales representative or distributor.

**Please note:** For more information about the configuration of the individual modules, refer to the related *Operating Instructions*.

Application	UltiMate 3000 System Module	Part No.
	SRD-3600—Solvent rack with 6-channel vacuum degasser (intended, e.g., for use with a DGP-3600)	5035.9230
	<i>—or—</i> SR-3000—Solvent rack for secure positioning of solvent bottles (without degasser, intended, e.g., for use with an LPG-3400)	5035.9245
	DGP-3600—Ternary dual low-pressure micro gradient pump (with loading pump and micro pump in one enclosure) Standard pump (DGP-3600M) Biocompatible pump (DGP-3600MB)	5035.0050 5037.0060
	<i>—or—</i> LPG-3400—Quaternary low-pressure micro gradient pump Standard pump (LPG-3400M) Biocompatible pump (LPG-3400MB)	5035.0045 5037.0055

Application	UltiMate 3000 System Module	Part No.
Nano HPLC	VWD-3100—Variable wavelength detector for one wavelength VWD-3400—Variable wavelength detector for four wavelengths with nano flow cell (must be ordered separately)	5074.0005 5074.0010 6074.0270
	FLM-3100(B) with two 10-port micro switching valves Standard flow manager (FLM-3100) Biocompatible flow manager (FLM-3100B) — <i>or</i> —	5720.0010 5721.0010
	FLM-3200(B) with 10-port micro and 6-port nano switching valves Standard flow manager (FLM-3200) Biocompatible flow manager (FLM-3200B) —or—	5720.0020 5721.0020
	FLM-3300(B) with one 10-port micro switching valve Standard flow manager (FLM-3300) Biocompatible flow manager (FLM-3300B)	5720.0030 5721.0030
	<b>Note:</b> These flow managers are fitted with a splitter cartridge for a split ratio of 1:1000.	
Capillary	VWD-3100—Variable wavelength detector for one wavelength VWD-3400—Variable wavelength detector for four wavelengths with capillary flow cell (must be ordered separately)	5074.0005 5074.0010 6074.0280
HPLC	FLM-3100(B) with two 10-port micro switching valves Standard flow manager (FLM-3100) Biocompatible flow manager (FLM-3100B) —or—	5720.0015 5721.0015
	FLM-3200(B) with 10-port micro and 6-port nano switching valves Standard flow manager (FLM-3200) Biocompatible flow manager (FLM-3200B) —or—	5720.0025 5721.0025
	FLM-3300(B) with one 10-port micro switching valve Standard flow manager (FLM-3300) Biocompatible flow manager (FLM-3300B)	5720.0035 5721.0035
	<b>Note:</b> These flow managers are fitted with a splitter cartridge for a split ratio of 1:100.	
Micro HPLC	VWD-3100—Variable wavelength detector for one wavelength VWD-3400—Variable wavelength detector for four wavelengths with micro flow cell (must be ordered separately)	5074.0005 5074.0010 6074.0290
	FLM-3100(B) with two 10-port micro switching valves Standard flow manager (FLM-3100) Biocompatible flow manager (FLM-3100B) —or—	5720.0018 5721.0018
	FLM-3200(B) with 10-port micro and 6-port nano switching valves Standard flow manager (FLM-3200) Biocompatible flow manager (FLM-3200B) —or—	5720.0028 5721.0028
	FLM-3300(B) with one 10-port micro switching valve Standard flow manager (FLM-3300) Biocompatible flow manager (FLM-3300B)	5720.0038 5721.0038
	<b>Note:</b> These flow managers are fitted with a splitter cartridge for a split ratio of 1:6.	

		1			
	VWD-3100—Variable wavelength detector for one wavelength	5074.0005			
	VWD-3400—Variable wavelength detector for four wavelengths	5074.0010			
Monolithic	with nano flow cell (must be ordered separately)	6074.0270			
HPLC	FLM-3100(B) with two 10-port micro switching valves				
	Standard flow manager (FLM-3100)	5720.0015			
	Biocompatible flow manager (FLM-3100B)	5721.0015			
	FLM-3200(B) with 10-port micro and 6-port nano switching valves				
	Standard flow manager (FLM-3200)	5720.0025			
	Biocompatible flow manager (FLM-3200B)	5721.0025			
	FLM-3300(B) with one 10-port micro switching valve				
	Standard flow manager (FLM-3300)				
	Biocompatible flow manager (FLM-3300B)	5721.0035			
	<b>Note:</b> These flow managers are fitted with a splitter cartridge for a split ratio of 1:100.				
	WPS-3000T(B)— Autosampler with temperature control				
	Standard autosampler (WPS-3000)T	5820.0010			
Biocompatible autosampler (WPS-3000TB)					
	<i>—or</i> —				
	WPS-3000(B)— Autosampler without temperature control				
	Standard autosampler (WPS-3000)	5820.0020			
	Biocompatible autosampler (WPS-3000B)	5821.0020			

#### 1.5 Intended Use

The UltiMate 3000 system is designed to perform equally well as system for routine analyses or as a sophisticated research instrument for use in capillary, nano, and micro HPLC (high performance liquid chromatography) applications.

The system is controlled by the **Chromeleon** Chromatography Management System. However, it can also be operated with other data systems, such as Analyst<sup>®</sup> (Applied Biosystems/MDS Sciex), HyStar<sup>TM</sup> (Bruker Daltonics), or Xcalibur<sup>®</sup> (Thermo Electron Corporation). To do so, installation of the DCMS<sup>Link</sup> (Dionex Chromatography Mass Spectrometry Link) software is required.

Please note that the system may be operated only using the accessories originally supplied with the system modules and within its technical specification ( $\rightarrow$  page 67).

If there is any question regarding appropriate usage, contact Dionex before proceeding.

Dionex cannot be held liable for any damage, material or otherwise, resulting from inappropriate or improper use of the system.

# 2 Installation

#### 2.1 Facility Requirements

After unpacking the modules of the UltiMate 3000 system, allow the units to warm up for approximately 4 hours before connecting them to the power supply. This delay allows any condensation that might have occurred during shipping to evaporate. After 4 hours, check the modules; if the condensation is still there, allow them to continue to warm up (without connecting them to the mains) until the condensation is completely gone.

Install the system in the laboratory on a stable surface that is free of vibration. Make sure that the surface is resistant to solvents. Avoid locations with extreme changes in temperature (such as direct sunlight or drafts) and high humidity. Allow sufficient clearance behind the system for power connections and ventilation.

Dionex recommends that you stack the individual modules of an UltiMate 3000 system, for example, as shown below. Note that the arrangement of the system modules depends on the application ( $\rightarrow$  Application Examples and Fluid Connections, page 13).



Fig. 1: Example for an UltiMate 3000 system

#### 2.2 Rear Panel Connectors



Fig. 2: Rear panel view of an UltiMate 3000 system

**Please note:** Fig. 2 illustrates how the system modules are interconnected. This applies accordingly to for any other system structure.

#### 2.2.1 Power Connection

Use the power cords from the accessories kits to connect the modules to the mains. The instruments are equipped with a standard power supply unit with an automatic voltage selector. Thus, no adjustment is required to adapt the line voltage to local voltage requirements.

**i** Please note: The SRD-3600 solvent rack is usually connected to the mains via the pump ( $\rightarrow$  Fig. 2). However, an external power supply unit (part no. 6510.0004) is available as an option.

#### 2.2.2 Interfaces for Device Control

Apart from the solvent rack, all modules of an UltiMate 3000 system can be connected to the server PC separately. However, Dionex recommends interconnecting the modules, and then connecting the system to the Chromeleon server PC via only one USB connection, e.g., from the autosampler ( $\rightarrow$  Fig. 2).

**Please note:** The SRD-3600 solvent rack can be operated with Chromeleon via the pump.

For information about the connection ports on the system modules, as well as the connector pinout, refer to the *Operating Instructions* for the respective instrument.

#### 2.3 Opening the Front Panel

To provide easy access to the components in the instrument compartments, the front panels of the autosampler, detector, pump, and solvent rack tilt upward. The open cover locks in its topmost position. The front panel of the flow manager tilts downward.

**M** Important: When lifting or moving an instrument, always lift by the bottom or sides. Lifting the instrument by the front panel may damage the front panel door.

▲ Important: Lorsque vous soulevez ou déplacez un instrument, saisissez la toujours par le dessous ou les côtés l'unité. Soulever l'unité par le panneau avant risque d'endommager la porte du panneau avant.

#### 2.4 Preparing the System

- ▲ Important: The system modules are filled with 2-propanol or an isopropyl alcohol-towater mixture when being shipped from the factory. During initial operation of the modules, make sure that the solvents used are miscible. Otherwise, use an appropriate intermediate solvent.
- ▲ Important: Les instruments dans un système UltiMate 3000 sont stockés sous 2propanol ou isopropanol et eau. Lors du démarrage initial des instruments, assurez-vous que les solvants utilisés soient miscibles. Sinon, suivez les étapes intermédiaires appropriées.
- Observe the information about the facility requirements when installing the system.
- Connect the drain tubing from the devices' accessories kits to the outlet of the modules to direct liquids that might have gathered in the enclosure to the waste. To do so, you must cut the tubing to appropriate lengths (see table below). The outlets are on the bottom right of the devices.
  - Please note: You may connect the tubing as shown in Fig. 3. To do so, use the connecting pieces from the devices' accessories kits.



Fig. 3: Connecting drain tubing

For the system structure shown in Fig. 3, Dionex recommends cutting the tubing to the following lengths:

Tubing from	Length (mm)
Solvent rack to pump	215
Pump to detector	133
Detector to flow manager	217
Flow manager to autosampler	272
Flow manager to Tee (for security loop)	140
Pump, detector or autosampler to Tee	30

Connect the drain tubing from the solvent rack to the pump and from the pump to the detector as shown in Fig. 4 a. Dionex recommends connecting the drain tubing from the flow manager as shown in Fig. 4 b. This is to avoid that the gas sensor in the flow manager is influenced by the drain tubing from the other modules. As an alternative, you may direct the drain tubing from the Tee of the flow manager separately to the waste container. Always connect the drain tubing from the bottom module as shown in Fig. 4 c.



Fig. 4: Connected drain tubing

- ▲ Important: The drain tube must remain below the drain port. If the drain tube is above the drain port, the liquid may flow back into the interior and cause damage to the instrument. Also, be careful not to use tubing that is bent.
- Important: Afin d'éviter d'endommager l'instrument, assurez-vous qu'aucune pièce des tubes n'est placée plus haute que le port de raccordement.



Wrong



Correct

**Please note:** In addition to the outlets described above, the flow manager and the solvent rack are equipped with another outlet on the rear panel. Also, the flow manager and the WPS-3000T autosampler with temperature control have an additional outlet at the bottom right ( $\rightarrow$  Fig. 3). Make sure to connect all outlets to the required tubing as described in the *Operating Instructions* for the devices!

- Prepare the individual modules as described in the *Operating Instructions* for the respective instrument.
- Connect the modules on the rear panel as described in Rear Panel Connectors ( $\rightarrow$  page 8).
- For examples of the fluid connections between the system modules, refer to Application Examples and Fluid Connections (→ page 13).
- Use the power cords shipped with the instruments to connect the units to the mains. Turn on the instruments by pressing the power switch on the rear panel.

### **3 Application Examples and Fluid Connections**

Different system configurations allow you to use the UltiMate 3000 system for various applications. For information about the related system setup and the fluid connections in the system, refer to:

- Manual Injection ( $\rightarrow$  page 16)
- **Direct Injection** ( $\rightarrow$  page 20)
- **Preconcentration** ( $\rightarrow$  page 24)
- **2D LC Salt Plugs** ( $\rightarrow$  page 28)
- Comprehensive 2D LC ( $\rightarrow$  page 33)
- Parallel LC ( $\rightarrow$  page 39)

When installing the fluid connections in an UltiMate 3000 system, keep the following in mind:

▲ Important:	Different fitting systems are used in an UltiMate 3000 system. Therefore, install the capillaries and fittings only at the positions for which they are intended.
⚠ Important:	Différents types de raccords sont utilisés dans le système UltiMate 3000. Par conséquent, installez les capillaires et les raccords uniquement dans aux endroits où ils sont prévus.
⚠ Important:	When you operate biocompatible system modules, observe the instructions for connecting the capillaries and other components. Refer to the related sections of the <i>Operating Instructions</i> for the module(s).
▲ Important:	Avant de accorder les capillaires où des autres éléments à un module biocompatible, référez-vous aux sections correspondantes dans le mode d'emploi pour le(s) module(s).
▲ Important:	Dionex recommends starting the fluid connections for the system modules at the pump. Connect the capillary to the pump first and rinse thoroughly before connecting the other end of the capillary to another module.
▲ Important:	Lorsque vous connectez les capillaires, respectez la séquence suivante: Raccordez le capillaire à la pompe. Rincez le capillaire avant de raccorder l'autre fin du capillaire à l'autre unité.
<b>▲</b> Important:	When connecting the capillaries, make sure that the connectors are free from contaminants. Even minute particles may cause damage to the flow splitter, flow control valve, and column.

▲ Important: Lorsque vous connectez les capillaires, assurez-vous que les raccords sont exempts de tout contaminant. Même d'infimes particules peuvent causer des dommages au système (ex. diviseur de débit, vanne de régulation de débit et colonne).

- **M** Important: Use only the capillaries shipped with the modules and original Dionex spare capillaries.
- **Mimportant:** Utilisez uniquement les capillaires fournis avec les unités et les capillaires de rechange d'origine Dionex.
- **Important:** To connect the capillaries to an injection valve or selector valve, install only the ferrules and fittings shipped with the capillaries.
- **Important:** Pour brancher les capillaires à une vanne d'injection ou de sélection, installez uniquement les ferrules et les raccords livrés avec la vanne.
- **Important:** Reuse used fittings and ferrules only for the same capillary connection. This is to avoid increased dead volume.
- **Important:** La réutilisation des raccords et ferrules n'est possible que pour la connexion capillaire d'origine, afin d'éviter l'apparition de volumes morts
- **1** Please note: The inlets of the pump's outlet units are fitted with frit holders and inline filters. The pump is shipped with steel frits with a porosity of  $0.5 \,\mu\text{m}$ . Check the permeability of the filter frits at regular intervals. When the pump delivers water at a flow rate of 2 ml/min and when the outlet is open, the pressure should not exceed 4 bar. If necessary, replace the filter frits (part no. 6000.0045 = 10 filter frits for DGP-3600M and LPG-3400M pumps or 6268.0002 for 10 filter frits for DGP-3600MB or LPG-3400MB pumps) as described in the *Operating Instructions* for the pump.
- **I** Please note: Keep in mind that the flow splitter in the flow manager, the flow cell in the detector and the associated capillaries depend on the specific application and that it might be necessary to replace them as necessary.

Description	Part No.
Splitter cartridge (standard) for nano HPLC applications (split ratio 1:1000)	6720.3150A
Splitter cartridge (standard) for nano HPLC applications (split ratio 1:300)	6720.3155A
Splitter cartridge (standard) for capillary HPLC applications (split ratio 1:100)	6720.3160A
Splitter cartridge (standard) for capillary HPLC applications (split ratio 1:15)	6720.3165A
Splitter cartridge (standard) for micro HPLC applications (split ratio 1:6)	6720.3170A
Splitter cartridge (biocompatible) for nano HPLC applications (split ratio 1:1000)	6721.3150A
Splitter cartridge (biocompatible) for nano HPLC applications (split ratio 1:300)	6721.3155A
Splitter cartridge (biocompatible) for capillary HPLC applications (split ratio 1:100)	6721.3160A
Splitter cartridge (biocompatible) for capillary HPLC applications (split ratio 1:15)	6721.3165A
Splitter cartridge (biocompatible) for micro HPLC applications (split ratio 1:6)	6721.3170A
Flow cell for nano HPLC applications	6074.0270
Flow cell for capillary HPLC applications	6074.0280
Flow cell for micro HPLC applications	6074.0290

The following flow splitters and flow cells are available:

For nano HPLC applications, the inner diameter of the capillaries is  $20 \ \mu m$ .

For capillary HPLC applications, the inner diameter is 50 µm.

For micro HPLC applications, the inner diameter is 75  $\mu$ m. If the back pressure generated by the connecting tubing is too high, tubing with an inner diameter of 130  $\mu$ m can be used.

For applications with Monolithic columns with an inner diameter of 100  $\mu$ m, the inner diameter of the connecting capillaries is 20  $\mu$ m.

For applications with Monolithic columns with an inner diameter of 200  $\mu$ m, the inner diameter of the connecting capillaries before the column is 50  $\mu$ m. After the column, Dionex recommends an inner diameter of 20  $\mu$ m.

#### 3.1 Manual Injection (Basic Configuration)

For this application, the sample is injected manually and directed to the separation column via the switching valve in the flow manager.

Dionex recommends that you stack the system modules as shown below:



Fig. 5: Module arrangement for manual injection

The system includes the modules listed in the table below. In addition, the appropriate Basic Configuration Kit ( $\rightarrow$  section 8.1, page 64) is required.

System Module	Part No.				
	Nano HPLC	Capillary HPLC	Micro HPLC	Monolith. HPLC	
SR-3000	5035.9200				
LPG-3400	5035.0045 (standard) or 5037.0055 (biocompatible)				
FLM-3300 Standard Biocompatible	5720.0030 5021.0030	5720.0035 5021.0035	5720.0038 5021.0038	5720.0035 5021.0035	
Basic Configuration Kit Standard Biocompatible	6720.0065 6721.0065	6720.0082 6721.0082	6720.0083 6721.0083	6720.0084 6721.0084	

Fig. 6 and the related table provide an overview of the fluid connections for manual injection. For an example of the fluid connections for nano HPLC, also refer to Fig. 7 ( $\rightarrow$  page 19).



Fig. 6: Fluid connections for manual injection (overview)

No.	Description	Part No. <sup>1</sup>			
		Nano HPLC	Capillary HPLC	Micro HPLC	Monolith. HPLC
1	Solvent lines (analytical)		6030	0.2548	
2	Solvent lines from degasser to proportioning valve (set of 3 including the appropriate fittings)		6035	5.2540	
3	Connection proportioning valve to working pump head (including fittings)		6035	5.2514	
4	Capillary from working pump head to equilibration pump head (including fittings)	603	5.3014 (standard) or 6	6037.3011 (biocomp	atible)
5	Capillary from equilibration head to outlet unit (including fittings)	603	5.3014 (standard) or 6	5037.3011 (biocomp	atible)
6	Inline filter (0.5 µm) in frit holder on outlet unit	6000.0045 (standard) or 6282.0022 (biocompatible)			atible)
7	Capillary from pump to flow splitter (short connection)	6035	.2553 <sup>2</sup> (standard) or 6	5037.2553 <sup>2</sup> (biocomp	patible)
8	Capillary from switching valve to flow splitter (both in flow manager) Standard Biocompatible	6720.0033 <sup>2</sup> 6721.0033 <sup>2</sup>	$6720.0034^{2}$ $6721.0034^{2}$	$6720.0035^{2}$ $6721.0035^{2}$	$6720.0034^{2}$ $6721.0034^{2}$
9	Capillary from port 6 to port 8 of the switching valve Standard Biocompatible	$\frac{6820.0015^2}{6821.0015^2}$	$\frac{6820.0016^2}{6821.0016^2}$	$\frac{6820.0018^2}{6821.0018^2}$	$\frac{6820.0015^2}{6821.0015^2}$
10	Connection from switching valve (flow manager) to separation column	Connect the column to the switching valve. If necessary, use the appropriate connection parts from the respective Basic Configuration kit.			
11	Separation column	160321 <sup>2</sup>	160295 <sup>2</sup>	160282 <sup>2</sup>	161409 <sup>2</sup>
12	Separation column outlet	All columns have an inbuilt outlet.			
13	Capillary for manual injection	Included in Manual Injection Port, part no. 6720.9007 <sup>2</sup> (standard) or 6721.9007 <sup>2</sup> (biocompatible)			

<sup>1</sup> The part number refers to the packing unit. For more information, contact your Dionex Sales Representative. <sup>2</sup> These parts are included in the Basic Configuration Kit for the related application ( $\rightarrow$  section 8.1, page 64).



The part numbers in Fig. 7 refer to nano HPLC (standard devices):

Fig. 7: Fluid connections for manual injection (nano HPLC, standard devices)

#### 3.2 Direct Injection

For this application, sample is drawn by the syringe through the needle and placed in the sample loop when the autosampler's injection valve is in **Load** position. The needle pair withdraws liquid from the well or vial and then descends into the wash port. The injection valve is switched to **Inject** position, directing the sample flow from the sample loop through the high-pressure circuit to the separation column.

Dionex recommends that you stack the system modules as shown below:



Fig. 8: Module arrangement for direct injection

The system includes the modules listed in the table below. In addition, the appropriate Direction Injection Kit ( $\rightarrow$  section 8.2, page 64) is required.

System Module	Part No.			
	Nano HPLC	Capillary HPLC	Micro HPLC	Monolith. HPLC
SR-3000		5035.9	9200	
LPG-3400	5035	.0045 (standard) or 50	)37.0055 (biocom	patible)
VWD-3100* VWD-3400*	5074.0005 5074.0010 *Please note that flow cells must be ordered separately, for order numbers see page 15.			
FLM-3300 Standard Biocompatible	5720.0030 5721.0030	5720.0035 5721.0035	5720.0038 5721.0038	5720.0035 5721.0035
WPS-3000 <i>or</i> WPS-3000 T		.0010 (standard) or 58 .0020 (standard) or 58	· · · · · · · · · · · · · · · · · · ·	1 /
Direct Injection Kit Standard Biocompatible	6720.0042 6721.0042	6720.0043 6721.0043	6720.0046 6721.0046	6720.0044 6721.0044

Fig. 9 and the related table provide an overview of the fluid connections for direct injection. For an example of the fluid connections for nano HPLC, also refer to Fig. 10 ( $\rightarrow$  page 23).



Fig. 9: Fluid connections for direct injection (overview)

No.	Description	Part No. <sup>1</sup>			
		Nano HPLC	Capillary HPLC	Micro HPLC	Monolith. HPLC
1	Solvent lines (analytical)	6030.2548			
2	Solvent lines from degasser to proportioning valve (set of 3 including the appropriate fittings)	6035.2540			
3	Connection proportioning valve to working pump head (including fittings)		6035	.2514	
4	Capillary from working pump head to equilibration pump head (including fittings)	6035.3014 (standard) or 6037.3011 (biocompatible)			
5	Capillary from equilibration head to outlet unit (including fittings)	6035.3014 (standard) or 6037.3011 (biocompatible)			
6	Inline filter (0.5 µm) in frit holder on outlet unit	6000.0045 (standard) or 6282.0022 (biocompatible)			
7	Capillary from pump to flow splitter Long connection (system with detector) short connection (system without detector)	6035.2550 <sup>2</sup> (standard) or 6037.2550 <sup>2</sup> (biocompatible) 6035.2553 <sup>2</sup> (standard) or 6037.2553 <sup>2</sup> (biocompatible)			
8	Capillary from separation column to detector flow cell	The flow cell is shipped with appropriate capillaries.			
9	Separation column	160321 <sup>2</sup>	160295 <sup>2</sup>	$160282^2$	161409 <sup>2</sup>
10	Capillary from autosampler switching valve to separation column (PEEKsil) Standard Biocompatible	6720.0024 <sup>2</sup> 6721.0024 <sup>2</sup>	$     6720.0025^{2} \\     6721.0025^{2} $	6720.0026 <sup>2</sup> 6721.0026 <sup>2</sup>	$6720.0025^{2}$ $6721.0025^{2}$
11	Capillary from autosampler switching valve to flow splitter (PEEKsil) Standard Biocompatible	$     6720.0027^2 \\     6721.0027^2 $	$6720.0028^{2}$ $6721.0028^{2}$	$     6720.0029^2      6721.0029^2 $	$6720.0028^{2}$ $6721.0028^{2}$
12	Standard needle (2.4 µl, fused silica)	6820.3010 (standard) or 6821.3010 (biocompatible)			
13	Sample loop Standard Biocompatible		.0015 <sup>2</sup> .0015 <sup>2</sup>	$\frac{6820.0018^2}{6821.0018^2}$	$\frac{6820.0015^2}{6821.0015^2}$

<sup>1</sup> The part number refers to the packing unit. For more information, contact your Dionex Sales Representative. <sup>2</sup> These parts are included in the Direct Injection Kit for the related application ( $\rightarrow$  section 0, page 64).



The part numbers in Fig. 10 refer to nano HPLC (standard devices):

Fig. 10: Fluid connections for direct injection (nano HPLC, standard devices)

P/N 6720.0024

WPS 3000

#### 3.3 Preconcentration

For this application, sample is drawn by the syringe through the needle and placed in the sample loop when the autosampler's injection valve is in **Load** position. The needle pair withdraws liquid from the well or vial and then descends into the wash port. The injection valve is switched to the **Inject** position, allowing the loading pump to direct the sample flow from the sample loop onto the trap column for sample enrichment. (For information about how to install the trap column, refer to section 3.7.) The switching valve in the flow manager is switched from **10-1** to **1-2**. The micro pump and the flow splitter direct the sample onto the separation column. Dionex recommends that you stack the system modules as shown below:



Fig. 11: Module arrangement for preconcentration

The system includes the modules listed in the table below. In addition, the appropriate Preconcentration Kit ( $\rightarrow$  section 8.3, page 65) is required:

System Module	Part No.				
	Nano HPLC	Capillary HPLC	Micro HPLC	Monolith. HPLC	
SRD-3600	600 5035.9230				
DGP-3600 5035.0050 (standard) or 5037.0060 (biocompatible)			npatible)		
VWD-3100* VWD-3400*	5074.0005 5074.0010 *Please note that flow cells must be ordered separately, for order numbers see page 15.				
FLM-3300 Standard Biocompatible	5720.0030 5721.0030	5720.0035 5721.0035	5720.0038 5721.0038	5720.0035 5721.0035	
WPS-3000 <i>or</i> WPS-3000 T	5820.0010 (standard) or 5821.0010 (biocompatible) 5820.0020 (standard) or 5821.0020 (biocompatible)				
Preconcentration Kit Standard Biocompatible	6720.0047 6721.0047	6720.0048 6721.0048	6720.0050 6721.0050	6720.0049 6721.0049	



Fig. 12 and the related table provide an overview of the fluid connections for preconcentration. For an example of the fluid connections for nano HPLC, also refer to Fig. 13 ( $\rightarrow$  page 27).

Fig. 12: Fluid connections for preconcentration (overview)

No.	Description	Part No. <sup>1</sup>			
		Nano HPLC	Capillary HPLC	Monolith. HPLC	
1	Solvent lines (analytical)	6030.2548			
2	Solvent lines from degasser to proportioning valve (set of 3 including the appropriate fittings)	6035.2540			
3	Connection proportioning valve to working pump head (including fittings)	6035.2514			
4	Capillary from working pump head to equilibration pump head (including fittings)	6035.3014 (standard) or 6037.3011 (biocompatible)			
5	Capillary from equilibration head to outlet unit (including fittings)	6035.3014 (standard) or 6037.3011 (biocompatible)			
6	Inline filter (0.5 µm) in frit holder on outlet unit	6000.0045 (standard) or 6282.0022 (biocompatible)			
7	Capillary from pump to flow splitter Long connection (system with detector) Short connection (system without detector)	6035.2550 <sup>2</sup> (standard) or 6037.2550 <sup>2</sup> (biocompatible) 6035.2553 <sup>2</sup> (standard) or 6037.2553 <sup>2</sup> (biocompatible)			
8	Capillary from flow splitter to flow manager switching valve Standard Biocompatible	$6720.0033^{2}$ $6721.0033^{2}$	$6720.0034^2$ $6721.0034^2$	$\frac{6720.0034^2}{6721.0034^2}$	
9	Trap column holder (with the appropriate capillaries) and Trap column	$\frac{6720.0012^2}{160454^2}$	$\frac{6720.0012^2}{160454^2}$	 163972 <sup>2</sup>	
10	Separation column (The column is connected directly to the switching valve.)	160321 <sup>2</sup>	160295 <sup>2</sup>	161409 <sup>2</sup>	
11	Capillary from the separation column to the detector flow cell	The flow cell is shipped with appropriate capillaries.			
12	Capillary from loading pump outlet to autosampler Long connection (system with detector) Short connection (system without detector)	6720.0032 <sup>2</sup> (standard) or 6721.0032 <sup>2</sup> (biocompatible) 6720.0031 (standard) or 6721.0031 (biocompatible)			
13	Capillary from autosampler to flow manager switching valve	6720.0038 <sup>2</sup> (standard) or 6721.0038 <sup>2</sup> (biocompatible)			
14	Sample loop (20 µl)	6820.0018 <sup>2</sup> (standard) or 6821.0018 <sup>2</sup> (biocompatible)			
15	Standard needle (2.4 µl, fused silica)	6820.3010 (standard) or 6821.3010 (biocompatible)			

<sup>1</sup> The part number refers to the packing unit. For more information, contact your Dionex Sales Representative. <sup>2</sup> These parts are included in the Preconcentration Kit for the related application ( $\rightarrow$  section 8.3, page 65).



The part numbers in Fig. 13 refer to nano HPLC (standard devices):

Fig. 13: Fluid connections for preconcentration (nano HPLC, standard devices)

#### 3.4 2D LC Salt Plug

This application supports online two-dimensional separation of peptides: While the autosampler's injection valve is in **Load** position, sample is drawn by the syringe through the needle and placed in the sample loop. The needle pair withdraws liquid from the well or vial and then descends into the wash port. The injection valve is switched to the **Inject** position, allowing the loading pump to direct the sample onto the ion exchange column (SCX). The right valve in the flow manager is in position **10-1**. The part of the sample that is not retained is trapped on the reverse phase trap column; the left valve in the flow manager is in position **10-1**. (For information about how to install the trap column, refer to section 3.7.)

When loading is finished, the ion exchange column is removed from the fluidic path by switching the right valve to position **1-2**. This allows desalting the sample collected on the trap column with the loading pump.

After the desalting step, the left valve is switched to position **1-2** to run the analytical gradient through the trap column and the reverse phase separation column.

When the first fraction is separated, both valves are switched to position **10-1** and a salt solution is injected. A new fraction of peptides elutes from the ion exchange column to the trap column. The desalting and analytical steps described above are carried out.

The process is iterated with increasing concentrations of salt injected until all the peptides from the ion exchange column are eluted and separated on the reverse phase column.

Dionex recommends that you stack the system modules as shown below:



Fig. 14: Module arrangement for 2D LC salt plug

The system includes the modules listed in the table below. In addition, the 2D LC Salt Plug Kit ( $\rightarrow$  section 8.4, page 65) is required:

System Module	Part No.		
	Nano HPLC		
SRD-3600	5035.9230		
DGP-3600	5035.0050 (standard) or 5037.0060 (biocompatible)		
VWD-3100* VWD-3400*	5074.0005 5074.0010		
	*Please note that flow cells must be ordered separately, for order numbers see page 15.		
FLM-3100	5720.0010 (standard) or 5721.0010 (biocompatible)		
WPS-3000 or WPS-3000 T	5820.0010 (standard) or 5821.0010 (biocompatible) 5820.0020 (standard) or 5821.0020 (biocompatible)		
2D LC Salt Plug Kit Standard Biocompatible	6720.0051 6721.0051		

Fig. 15 and the related table provide an overview of the fluid connections for 2D LC salt plug. Also, refer to Fig. 16 ( $\rightarrow$  page 32).



Fig. 15: Fluid connections for 2D LC salt plug (overview)

No.	Description	Part No. <sup>1</sup>		
		Nano HPLC		
1	Solvent lines (analytical)	6030.2548		
2	Solvent lines from degasser to proportioning valve (set of 3 including the appropriate fittings)	6035.2540		
3	Connection proportioning valve to working pump head (including fittings)	6035.2514		
4	Capillary from working pump head to equilibration pump head (including fittings)	6035.3014 (standard) or 6037.3011 (biocompatible)		
5	Capillary from equilibration head to outlet unit (including fittings)	6035.3014 (standard) or 6037.3011 (biocompatible)		
6	Inline filter (0.5 µm) in frit holder on outlet unit	6000.0045 (standard) or 6282.0022 (biocompatible)		
7	Capillary from pump to flow splitter Long connection (system with detector) Short connection (system without detector)	6035.2550 <sup>2</sup> (standard) or 6037.2550 <sup>2</sup> (biocompatible) 6035.2553 <sup>2</sup> (standard) or 6037.2553 <sup>2</sup> (biocompatible)		
8	Capillary from flow splitter to left switching valve in flow manager	6720.0033 <sup>2</sup> (standard) or 6021.0033 (biocompatible)		
9	Trap column holder (with appropriate capillaries) and Trap column	$\frac{6720.0012^2}{160454^2}$		
10	Separation column (The column is connected directly to the switching valve.)	160321 <sup>2</sup>		
11	Capillary from separation column to detector flow cell	The flow cell is shipped with appropriate capillaries.		
12	PEEKsil bridge from left to right switching valve in the flow manager	6720.0060 <sup>2</sup> (standard) or 6721.0060 <sup>2</sup> (biokompatibel)		
13	Ion exchange column (SCX)	162152 <sup>2</sup>		
14	Capillary from loading pump outlet unit to autosampler Long connection (system with detector) Short connection (system without detector)	6720.0032 <sup>2</sup> (standard) or 6721.0032 <sup>2</sup> (biocompatible) 6720.0031 (standard) or 6721.0031 (biocompatible)		
15	Sample loop (20 µl)	6820.0018 <sup>2</sup> (standard) or 6821.0018 <sup>2</sup> (biocompatible)		
16	Capillary from autosampler to right switching valve in flow manager	6720.0038 <sup>2</sup> (standard) or 6721.0038 <sup>2</sup> (biocompatible)		
17	Standard needle (2.4 µl, fused silica)	6820.3010 (standard) or 6821.3010 (biocompatible)		

<sup>1</sup> The part number refers to the packing unit. For more information, contact your Dionex Sales Representative. <sup>2</sup> These parts are included in the 2D LC Salt Plug Kit ( $\rightarrow$  section 8.4, page 65).



Fig. 16, too, illustrates the fluid connections for 2D LC salt plug. The part numbers in the picture refer to the standard devices.

Fig. 16: Fluid connections for 2D LC salt plug (nano HPLC, standard devices)
## 3.5 Comprehensive 2D LC

This application supports online comprehensive two-dimensional separation of peptides: While the autosampler's injection valve is in **Load** position, sample is drawn by the syringe through the needle and placed in the sample loop. The needle pair withdraws liquid from the well or vial and then descends into the wash port. The injection valve is switched to the **Inject** position, allowing the capillary pump to direct the sample to the ion exchange column (SCX).

The peptides that are not retained are collected on trap column 1 ( $\rightarrow$  Fig. 18, no. 12) that is inline with the ion exchange column. Valve 2 is in position **1-2**. In parallel, valve 1 is in position **1-2** to flush the trap column 2 ( $\rightarrow$  Fig. 18, no. 15) via the loading pump. (For information about how to install the trap columns, refer to section 3.7.)

At the end of the loading step, valve 2 is switched to position **10-1**, setting trap column 2 in line with the ion exchange column. Trap column 1 is now in line with the loading pump for a desalting step. When desalting is finished, valve 1 is switched to position **10-1**. This connects trap column 1 and the reverse phase column, thus allowing the separation of the peptides collected on trap column 1. During that time, a salt gradient is started. The peptides eluting from the ion exchange column are trapped on trap column 2, collecting a new peptides fraction.

At the end of the separation on the reverse phase column, valve 1 is switched to position **1-2** to condition trap column 1 via the loading pump (acetonitrile is removed). When trap column 1 is ready again, valve 2 is switched to position **1-2**. Trap column 1 is flushed with the salt buffer while trap column 2 is equilibrated with the loading solvent.

At this stage, a new salt gradient starting from the highest salt percentage reached in the previous one can be started to load peptides on trap column 1. Simultaneously, the peptides previously collected on the trap column 2 are being separated, with valve 1 in position **10-1**.

The process can be iterated, running an increasing salt concentration on the ion exchange column. In this way, peptides are collected on one trap column while a reverse phase separation is performed for the peptides collected on the other trap column during the previous iteration.

- **I** Please note: The right choice of the time allowed for desalting and reconditioning the trap columns is crucial for this application.
- Please note: The application comprises 2 pumps (LPG-3400 and DGP-3600) and two FLM-3300 flow managers ( $\rightarrow$  Fig. 17, page 34). Therefore, adapt the names under which the devices are identified in the installation environment and in the Chromeleon client in the Chromeleon Server Configuration program. Keep in mind that you may have to adapt the links to the associated control panels as well.



Dionex recommends that you arrange the system modules as shown below:

Fig. 17: Module arrangement for comprehensive 2D LC

The system includes the modules listed in the table below. In addition, the Comprehensive 2D LC Kit ( $\rightarrow$  section 8.5, page 65) is required:

System Module	Part No.	
	Capillary/Nano HPLC	
SR-3000	5035.9200	
LPG-3400	5035.0045 (standard) 5037.0055 (biocompatible)	
WPS-3000 or WPS-3000 T	5820.0010 (standard) or 5821.0010 (biocompatible) 5820.0020 (standard) or 5821.0020 (biocompatible)	
SRD-3600	5035.9230	
DGP-3600	5035.0050 (standard) or 5037.0060 (biocompatible)	
VWD-3100* VWD-3400*	5074.0005 5074.0010	
	*Please note that flow cells must be ordered separately, for order numbers see page 15.	
FLM-3300 (Nano)	5720.0030 (standard) or 5721.0030 (biocompatible)	
FLM-3300 (Capillary)	5720.0035 (standard) or 5721.0035 (biocompatible)	
Comprehensive 2D LC Kit	6720.0053 (standard) or 6721.0053 (biocompatible)	



Fig. 18 and the related table provide an overview of the fluid connections for 2D LC comprehensive for capillary (1<sup>st</sup> dimension) nano (2<sup>nd</sup> dimension) HPLC. Also, refer to Fig. 19 ( $\rightarrow$  page 38).

Fig. 18: Fluid connections for comprehensive 2D LC (overview)

No.	Description	Part No. <sup>1</sup>
		Capillary/Nano HPLC
1	Solvent lines (analytical)	6030.2548
2	Solvent lines from degasser to proportioning valve (set of 3 including the appropriate fittings)	6035.2540
3	Connection proportioning valve to working pump head (including fittings)	6035.2514
4	Capillary from working pump head to equilibration pump head (including fittings)	6035.3014 (standard) or 6037.3011 (biocompatible)
5	Capillary from equilibration head to outlet unit (including fittings)	6035.3014 (standard) or 6037.3011 (biocompatible)
6	Inline filter (0.5 µm) in frit holder on outlet unit	6000.0045 (standard) or 6282.0022 (biocompatible)
7	Capillary from capillary pump (DGP-3600) to flow splitter Long connection (system with detector) Short connection (system without detector)	6035.2556 <sup>2</sup> (standard) or 6037.2556 (biocompatible) 6035.2554 <sup>2</sup> (standard) or 6037.2554 (biocompatible)
8	Capillary from autosampler to flow splitter (cap or micro)	6720.0028 <sup>2</sup> (standard) or 6721.0028 (biocompatible)
9	Capillary from autosampler to ion exchange column (SCX)	6720.0058 <sup>2</sup> (standard) or 6721.0058 <sup>2</sup> (biocompatible)
10	Ion exchange column (SCX)	162122 <sup>2</sup>
11	Capillary from ion exchange column (SCX) to switching valve	The ion exchange column has an inbuilt outlet.
12	Trap column holder (with appropriate capillaries) and Trap column	$\frac{6720.0012^2}{160454^2}$
13	Capillary from pump loading pump (LPG-3400) to switching valve (nano FLM)	6720.0057 <sup>2</sup> (standard) or 6721.0057 <sup>2</sup> (biocompatible)
14	PEEKsil bridges from switching valve (nano FLM) to switching valve (cap/micro FLM)	(2x) 6720.0062 <sup>2</sup> (standard) or 6721.0062 <sup>2</sup> (biocompatible)
15	Trap column holder (with appropriate capillaries) and Trap column	$\frac{6720.0012^2}{160454^2}$
16	PEEKsil bridge: Nano flow manager: from port 4 to port 7 Cap/micro flow manager: from port 3 to port 8	6720.0061 <sup>2</sup> (standard) or 6721.0061 <sup>2</sup> (biocompatible) 6720.0061 <sup>2</sup> (standard) or 6721.0061 <sup>2</sup> (biocompatible)
17	Separation column (The column is connected directly to the switching valve.)	160321 <sup>2</sup>
18	Capillary from separation column to detector flow cell	The flow cell is shipped with appropriate capillaries.

No.	Description	Part No. <sup>1</sup>	
		Capillary/Nano HPLC	
19	Capillary from analytical pump (DGP-3600) to flow splitter Long connection (system with detector) Short connection (system without detector)	6035.2556 <sup>2</sup> (standard) or 6037.2556 (biocompatible) 6035.2554 <sup>2</sup> (standard) or 6037.2554 (biocompatible)	
20	Capillary from nano flow splitter to switching valve	6720.0033 <sup>2</sup> (standard) or 6021.0033 (biocompatible)	
21	Sample loop (20 µl)	6820.0018 <sup>2</sup> (standard) or 6821.0018 <sup>2</sup> (biocompatible)	
22	Standard needle (2.4 µl, fused silica)	6820.3010 (standard) or 6821.3010 (biocompatible)	

<sup>1</sup> The part number refers to the packing unit. For more information, contact your Dionex Sales Representative. <sup>2</sup> These parts are included in the Comprehensive 2D LC Kit ( $\rightarrow$  section 8.5, page 65).

Fig. 19, too, illustrates the fluid connections for comprehensive 2D LC for capillary  $(1^{st}$  dimension) and nano  $(2^{nd}$  dimension) HLPC. The part numbers in the picture refer to the standard devices.



Fig. 19: Fluid connections for comprehensive 2D LC (standard devices)

## 3.6 Parallel LC

This application allows you to increase the throughput for peptide separations. While the autosampler's injection valve is in **Load** position, sample is drawn by the syringe through the needle and placed in the sample loop. The needle pair withdraws liquid from the well or vial and then descends into the wash port. The injection valve is switched to the **Inject** position, allowing the loading pump to direct the sample from the sample loop onto trap column 1 (in the FLM-3200;  $\rightarrow$  Fig. 21, no. 9) for sample preconcentration. (For information about how to install the trap columns, refer to section 3.7.)

Valve 1 (left switching valve in the FLM-3200) is in position **10-1**. In parallel, valve 2 (in the FLM-3300) is in position **1-2** to allow the wash step to elute remaining hydrophobic compounds from trap column 2 (in the FLM-3300;  $\rightarrow$  Fig. 21, no. 11) and separation column 2 (in the FLM-3300;  $\rightarrow$  Fig. 21, no. 19). The nano valve (right switching valve in the FLM-3200) is in position **6-1** to direct the eluent from the wash-step to the detector.

When the sample is loaded on trap column 1, valve 1 is switched to position 1-2. The analytical pump 1 then directs the sample from trap column 1 to separation column 1 (in the FLM-3200;  $\rightarrow$  Fig. 21, no. 14). At the time when the first peaks are expected, the nano valve is switched to position 1-2 to direct the eluent to the detector. In the meantime, separation column 2 is reconditioned. Shortly before the separation ends, valve 2 is switched to position 10-1. Trap column 2 is conditioned with the loading solvent.

As soon as the gradient over separation column 1 is finished, a new sample is injected. Valve 1 is in position **1-2.** Valve 2 is in position **10-1** to load the sample on trap column 2. At the same time, separation column 1 is washed and its eluent is collected, with the nano valve being in position **1-2**.

When the trap column 2 is loaded, value 2 is switched to position 1-2. This connects the separation column 2 with the trap column 2 (analytical pump). At the end of the wash step for separation column 1, the nano value is switched to position 6-1. The separation column 1 is reconditioned while the separation runs on separation column 2. Before the experiment ends, value 1 is switched to position 10-1 to prepare for the next injection.

This sequence, which can be repeated as often as needed, allows running the analysis on one column and simultaneously washing and equilibrating the other column.

**Please note:** The application comprises two pumps (LPG-3400 and DGP-3600) and two flow managers (FLM-3200 and FLM-3300;  $\rightarrow$  Fig. 20, page 40). Therefore, adapt the names under which the devices are identified in the installation environment and in the Chromeleon client in the Chromeleon Server Configuration program. Keep in mind that you may have to adapt the links to the associated control panels as well.



Dionex recommends that you arrange the system modules as shown below:

Fig. 20: Module arrangement for parallel LC

The system includes the modules listed in the table below. In addition, the appropriate Parallel LC Kit ( $\rightarrow$  section 8.6, page 66) is required:

System Module	Part No.		
	Nano/Nano HPLC	Capillary/Capillary HPLC	Monolith./Monolith. HPLC
SR-3000		5035.9200	
LPG-3400	5035.0045 (s	standard) or 5037.0055 (bioc	compatible)
WPS-3000 or WPS-3000 T		standard) or 5821.0010 (bioc standard) or 5821.0020 (bioc	
SRD-3600		5035.9230	
DGP-3600	5035.0050 (standard) or 5037.0060 (biocompatible)		
VWD-3100* VWD-3400*	5074.0005 5074.0010		
	*Please note that flow cells must be ordered separately, for order numbers see page 15.		
FLM-3200 Standard Biocompatible	5720.0020     5720.0025     5720.0025       5721.0020     5721.0025     5721.0025		
FLM-3300 Standard Biocompatible	5720.0030     5720.0035     5720.0035       5721.0030     5721.0035     5721.0035		
Parallel LC Kit Standard Biocompatible	6720.0054 6721.0054	6720.0055 6721.0055	6720.0056 6721.0056



Fig. 21 and the related table provide an overview of the fluid connections for parallel LC. For an example of the fluid connections for nano HPLC, also refer to Fig. 22 ( $\rightarrow$  page 44).

Fig. 21: Fluid connections for parallel LC (overview)

No.	Description		Part No. <sup>1</sup>	
		Nano/Nano HPLC	Capillary/Capillary HPLC	Monolith./Monolith. HPLC
1	Solvent lines (analytical)		6030.2548	
2	Solvent lines from degasser to proportioning valve (set of 3 including the appropriate fittings)		6035.2540	
3	Connection proportioning valve to working pump head (including fittings)		6035.2514	
4	Capillary from working pump head to equilibration pump head (including fittings)	6035.3014	(standard) or 6037.3011 (bio	ocompatible)
5	Capillary from equilibration head to outlet unit (including fittings)	6035.3014	(standard) or 6037.3011 (bic	ocompatible)
6	Inline filter (0.5 $\mu$ m) in frit holder on outlet unit	6000.0045	(standard) or 6282.0022 (bic	ocompatible)
7	Capillary from loading pump (LPG-3400) to autosampler	$6720.0057^2$	(standard) or 6721.0057 <sup>2</sup> (bi	ocompatible)
8	Capillary from autosampler to FLM-3200 left switching valve	6720.0038 <sup>2</sup> (standard) or 6721.0038 <sup>2</sup> (biocompatible)		
9	Trap column holder (with appropriate capillaries) and Trap column	$\frac{6720.0012^2}{160454^2}$	$\frac{6720.0012^2}{160454^2}$	 164972 <sup>2</sup>
10	PEEKsil bridge from left switching valve (FLM-3200) to FLM-3300 switching valve	e $6720.0059^2$ (standard) or $6721.0059^2$ (biocompatible)		ocompatible)
11	11 Trap column holder (with appropriate capillaries) and Trap column 6720.0012 <sup>2</sup> 160454 <sup>2</sup>		$\frac{6720.0012^2}{160454^2}$	164972 <sup>2</sup>
12	Capillary from analytical pump 1 (DGP-3600-left pump) to FLM-3200 flow splitter Long connection (system with detector) Short connection (system without detector)	$6035.2556^2$ $6035.2554^2$	(standard) or 6037.2556 <sup>2</sup> (bi (standard) or 6037.2554 <sup>2</sup> (bi	iocompatible) iocompatible)
13	FLM-3200: Capillary from flow splitter to left switching valve Standard Biocompatible	$6720.0033^{2}$ $6721.0033^{2}$	$\frac{6720.0034^2}{6721.0034^2}$	$\frac{6720.0034^2}{6721.0034^2}$
14	Separation column (The column is connected directly to the switching valve.)	160321 <sup>2</sup>	160295 <sup>2</sup>	16409 <sup>2</sup>
15	FLM-3200: Capillary from the separation column to the right switching valve	Connect the column to the switching valve. If necessary, use the appropriate connection parts from the respective Parallel LC kit.		
16	Capillary from right switching valve (FLM-3200) to detector flow cell	The flow ce	ell is shipped with appropriat	te capillaries.

No.	Description	Part No. <sup>1</sup>		
		Nano/Nano HPLC	Capillary/Capillary HPLC	Monolith./Monolith. HPLC
17	Capillary from analytical pump 2 (DGP-3600, right pump) to FLM-3300 flow splitter Long connection (system with detector) Short connection (system without detector)		(standard) or 6037.2556 (bi (standard) or 6037.2554 (bi	
18	FLM-3300: Capillary from flow splitter to switching valve Standard Biocompatible	$6720.0033^{2}$ $6721.0033^{2}$	$6720.0034^2$ $6721.0034^2$	$6720.0034^2$ $6721.0034^2$
19	Separation column (The column is connected directly to the switching valve.)	160321 <sup>2</sup>	160295 <sup>2</sup>	16409 <sup>2</sup>
20	Connection of separation column (FLM-3300) to right switching valve (FLM-3200)	The separation columns have an inbuilt fused silica outlet (for direct connection to the switching valve).		
21	Sample loop (20 µl)	6820.0018 <sup>2</sup> (standard) or 6821.0018 <sup>2</sup> (biocompatible)		
22	Standard needle (2.4 µl, fused silica)	6820.3010 (standard) or 6821.3010 (biocompatible)		ocompatible)

<sup>1</sup> The part number refers to the packing unit. For more information, contact your Dionex Sales Representative. <sup>2</sup> These parts are included in the Parallel LC Kit for the related application ( $\rightarrow$  section 8.6, page 66).



The part numbers in Fig. 22 refer to nano HPLC (standard devices):

Fig. 22: Fluid connections for parallel LC (nano HPLC; standard devices)

## 3.7 Installing a Trap Column

Various applications (e.g., Preconcentration, 2D LC Salt Plugs, Comprehensive 2D LC, Parallel LC) require the installation of one or two trap columns. When installing a trap column, make sure that the connections are made with no dead (delay) volume, as dead volume will cause a delay in the elution of the compounds of interest and lead to poor reproducibility.

The following problems indicate that dead volume has been introduced into the system:

- The injection profile slowly returns to baseline (see Fig. 23)
- The retention times (with known sample) shift to later elution
- The column pressure drop is larger than 10 bar
- Please note: The pressure stability option of the UltiMate 3000 system ("Hold pressure" mode) maintains a constant pressure during valve switching, however, it is not designed to compensate for dead volumes. Any dead volume will therefore cause a pressure drop in the column pressure trace.

Fig. 23 represents the chromatogram of a separation with zero dead volume (black line) and the effect of dead volume on the separation (blue line).



Fig. 23: Effect of Dead Volume on the Injection Profile and Separation

To establish a zero dead volume fitting connection when installing the trap column, follow the steps below. Both connecting tubings use a PEEK nut and ferrule at the trap column side, and a one-piece PEEK fingertight is required to connect the trap column to the valve (see Fig. 24).



Fig. 24: The Trap Column System

To connect the trap column:

- 1. Insert a trap column cartridge in the holder.
- 2. Slide the ferrule of each (pre-assembled) connecting tubing approximately 3 mm (0.1 inch) back on the tubing to extend the length of the tubing that enters the trap column.
- 3. Install the connecting tubing in the holder and tighten both fingertight fittings simultaneously. The ferrules will slide in place and no dead volume will be created in the connections. The O-rings will help to center the holder.
- 4. Connect the other ends of the connecting tubing to the switching valve. Make sure that the connection is also free of dead volume.

If the chromatogram indicates that dead volume is present, repeat the above process.

**Please note**: Make sure that all connections are leak-free and finger-tight.

# 4 Automated Control by Chromeleon

**Please note**: The descriptions below refer to Chromeleon 6.80.

## 4.1 General

The modules of the UltiMate 3000 system can be controlled by the Chromeleon Chromatography Management System. To control the modules, a suitable Chromeleon version (e.g., 6.50 Service Pack 10, 6.60 Service Pack 6, 6.70 Service Pack 3, or 6.80) and a Timebase Class 1 Chromeleon license are required. (If you have any questions, please contact your Dionex sales representative.)

Dionex recommends that you follow the installation steps below:

- Install the Chromeleon software before connecting the system modules to the USB port on the Chromeleon server PC. This will ensure that most of the necessary USB drivers are copied to appropriate locations.
- Connect the solvent rack to the 15-pin D-sub port on the pump.
- Connect the modules to each other. Use the USB cables that are shipped with the modules. Dionex recommends connecting all modules to one "central module" (e.g. the autosampler) and connecting this module to the Chromeleon Server PC ( $\rightarrow$  Fig. 2, page 8).
- Power up the "central module". The Plug&Play Manager (Hardware Wizard) guides you through the remaining USB installation for this module. Continue with powering up and installing the other USB modules. For more information about the USB installation steps, refer to the Operating Instructions for the modules.
- Install and configure the system modules in the Chromeleon Server Configuration program as described in the *Operating Instructions* for the module. You can add the modules in any order to the timebase. However, some settings affect more than one module. Therefore, the preferred order of installation is as follows (→ Fig. 25, page 48):
  - Flow Manager (FLM-3x00)
  - Pump (DGP-3600M or MB, LPG-3400M or MB):
  - Assign a flow splitter in the flow manager to a pump ( $\rightarrow$  section 4.2, page 49).
  - Autosampler (WPS-3000):
  - Synchronize the injection with the pump ( $\rightarrow$  section 4.4, page 53).
  - Detector (VWD-3x00)
  - Solvent Rack (SRD-3x00): No configuration needed

If you install the modules in a different order, reopen the pump's and autosampler's **Properties** pages and make the necessary configuration changes.



Fig. 25: Installation of an UltiMate 3000 system in the Server Configuration program (here: for Preconcentration, or 2D LC Salt Plug)

**Please note:** The *Operating Instructions* for the modules also provide an overview of the different commands and properties supported by Chromeleon for the respective instrument. For more information, refer to the *Chromeleon online Help* and the *Chromeleon manuals*.

**Please note:** Operational and/or Performance Qualification allows you to check and document the quality of your HPLC system. All required materials and detailed instructions are available from Dionex on request.

## 4.2 Assigning a Flow Splitter to a Pump

If your system includes an FLM-3000 series flow manager, you can use the **Devices** tab page of the pump's properties to specify whether a flow splitter is connected to the pump.

DGP-3600 Pump				×
Left Pump General	Left Solvents Devices	Relays Right Pu	Inputs Imp	Error Levels
Device Name	e: Pump			
Left Pump:	LoadingPump	on	Pumps	•
	uses FLM-3x00 Flow	Splitter <n< th=""><th>one&gt;</th><th>•</th></n<>	one>	•
	purges via WPS-300	OSL <	one>	•
Right Pump:	MicroPump	on	Pumps	•
	uses FLM-3x00 Flow	Splitter FLN	43x00_FLOWS	SPLITTER_1
	purges via WPS-300	OSL KN	one>	•
🔽 Share elu	ent bottles			
🔽 Share wa	ste bottle			
Pressure	Signal(s)			
			1	
	OK	Cancel	Apply	Help

*Fig. 26: Devices tab page (here: for a DGP-3600M)* 

**I** Please note: Flow splitter assignment is possible only if both the pump and the flow manager are installed in Chromeleon.

The default entry is **<None>**. To change the setting, click the arrow next to the **uses FLM-3x00 Flow Splitter** input field and select the splitter from the drop-down list.

**Please note:** When you assign a flow splitter to a pump, the pump flow settings apply directly to the flow on the splitter outlet, i.e., the split ratio is considered automatically.

**Please note:** For the standard configuration of the UltiMate 3000 system with a DGP-3600M pump, Dionex recommends assigning the flow splitter to the right pump (MicroPump).

Flow splitter assignment also affects the properties that are available for the pump in the **Commands** dialog box:

Chromeleon	Description	
Flow and/or	Flow rate in µl/min	
MasterFlow	Pumps without flow splitter assignment:	
	Flow indicates the total flow through the pump.	
	MasterFlow is not available.	
	Pumps with flow splitter assignment:	
	Flow indicates the flow through the column.	
	MasterFlow indicates the flow through the master pump, i.e., the total	
	flow which is the flow before the flow splitter.	
Pressure and/or	Indicates the pressure	
MasterPressure <u>Pumps without flow splitter assignment:</u>		
	Pressure indicates the pump pressure.	
	MasterPressure is not available.	
Pumps with flow splitter assignment:		
	Pressure indicates the current column pressure.	
	MasterPressure indicates the pressure of the master pump, i.e., the	
	pressure before the flow splitter.	

**Please note:** The **purges via WPS-3000SL** option is only available when using an autosampler with split-loop injection (WPS-3000(T)SL)..

For more information, refer to the *Operating Instructions* for the pump and/or flow manager or to the Chromeleon *Online Help*.

## 4.3 Recording the Pump Pressure as a Separate Channel

If your system includes a flow manager, the **Pressure Signal** check box is selected by default on the **Configuration** tab page in the flow manager's properties.

Accept this setting if you want to record the column pressure as a separate channel.

FLM-3x00 Flow Manager	×
General Components Configuration Relays Inputs Error Levels	
✓ Temperature Signal ColumnOven_Temp   ✓ Pressure Signal ColumnPressure	
Pressure <u>U</u> nit: bar Additional Signals Flow Control	
OK Cancel Apply Help	

Fig. 27: Configuration tab page

**Please note**: When your UltiMate 3000 system includes *one* flow manager: Accept the default name (**ColumnPressure**), under which the column pressure signal is identified in the installation environment.

When your system includes *more than one* flow manger: Adapt the name for all additional flow managers according to your application. Keep in mind that you have to re-link the corresponding controls on the related control panel(s).

 Please note: Select the Flow Control check box only if you want to perform Operational Qualification. When the check box is selected, additional channels are recorded. The additional values provide valuable information to Dionex Service for troubleshooting in case the flow manager does not pass Operational Qualification successfully. If the column pressure is recorded as a separate channel, **ColumnPressure** appears in the **Commands** dialog box, also.

Chromeleon	Description	
ColumnPressure	Click the "+"sign beside the name to display the items underneath:	
	<b>Delta</b> —reports the signal's slope, i.e., the difference between the current value and the value one second ago. This is useful for triggers.	
	<b>Signal</b> -has the following entries: Value-reports the current signal value (read-only), UpperLimit-sets the upper signal limit, and LowerLimit-sets the lower signal limit.	
	AcqOn–starts data acquisition. AcqOff–terminates data acquisition.	
	<b>Retention</b> –reports the retention time of the signal (read-only).	
	<b>MaxAutoStep</b> –sets the maximum step rate for Auto Step Mode (range: 0.15.1 s; default: 5.1 s).	
	<b>Step</b> -sets the step for data acquisition (range: 0.014.80 s; Auto selects the best step dynamically.)	
	Average–averages all measured values over the step interval. (The default setting is: On. Off records only the last point of each interval.)	

For more information, refer to the *Operating Instructions* for the flow manager and/or to the Chromeleon *Online Help*.

## 4.4 Synchronizing the Autosampler with a Pump

If your system includes a WPS-3000 or WPS-3000T autosampler, you can synchronize the injection command of the autosampler with the strokes of a DGP-3600 or LPG-3400 pump. Synchronization ensures that all injections are performed at the same phase of the pump cycle.

On the **Segments & Pump Link** tab page of the autosampler's properties, determine which synchronization is performed.

**Please note**: Dionex recommends always enabling synchronization as synchronization helps enhancing the analytical results considerably.

Click the arrow in the **Pump Link** section and select an option from the drop-down list:

WPS-3000 Autos	ampler 🗙
General Sharing	Segments & Pump Link Options Relays Inputs Error Levels
Well Plate 9	Sampler Segment Types
Red	40 Standard Vials
Green	40 Standard Vials
Blue	40 Standard Vials
Warning: B	Ensure that these settings match the installed items!
Pump Link-	
· · ·	gh Sampler is delivered by pump:
<none></none>	
<none></none>	PL STRK (DGP-3600 Pump #2)
UM3PUM	P_R_STRK ( DGP-3600 Pump #2 )
ТОМЗРОМ	P_STROKE (LPG-3x00 Pump #2)
	OK Cancel Apply Help

Fig. 28: Segments tab page

(here: when the UltiMate 3000 system includes an DGP-3600 pump)

**Please note**: The options are available only if both the autosampler and the pump are installed in Chromeleon.

- Select None to disable synchronization.
- When the UltiMate 3000 system includes a DGP-3600 pump: Select UM3PUMP \_L\_STRK to enable synchronization with the left pump. To enable synchronization with the right pump, select UM3PUMP \_R\_STRK.
- When the UltiMate 3000 system includes an LPG-3400 pump: Select **UM3PUMP\_STROKE** to enable synchronization.
- Please note: You can change the default synchronization assignment for a specific application, using the **PumpDevice** and **SyncWithPump** properties in the **Commands** dialog box under **Sampler** on the control panel or in the program file (PGM).

To disable synchronization for a specific application, set **SyncWithPump** to **Off**. Use the **PumpDevice** property to select the pump for which synchronization shall be performed. (The drop-down list contains names of the pumps installed in the Server Configuration program.)

Changing the settings for these properties from the **Commands** dialog box does not change the standard synchronization setting on the **Segments** tab page.

For more information, refer to the *Operating Instructions* for the autosampler and/or pump or to the Chromeleon *Online Help*.

### 4.5 Displaying the Degasser-Related Parameters

If your system includes an SRD-3x00 solvent rack, the degasser can be controlled with Chromeleon via the pump. In this case, the following degasser-related parameters are listed in the **Commands** dialog box under the pump to which the solvent rack is connected:

Chromeleon	Description
Degasser	Turns the solvent rack degasser on or off. For pumps operated with an SRD-3000 solvent rack, the setting should always be <b>On</b> . For pumps that are operated without degasser or with an external third-party degasser, select <b>Off</b> .
DegasserVacuum	Indicates the status of the degasser vacuum in the solvent rack ( <b>OK</b> or <b>NotOK</b> ; read-only).
SolventRackLeak	Indicates the status of the leak sensor in the solvent rack (Leak or NoLeak; read-only).

**Please note:** Dionex recommends always leaving the degasser on. Turning off the pump automatically turns off the degasser, too.

# 5 Soft Key Menus

For all UltiMate 3000 Nano and Cap system modules except the solvent rack, four soft keys under the front panel display provide access to various menus, allowing you to change certain settings and/or perform certain commands.

A magnetic Dionex menu pen (part no. 6300.0100) is included in each module's accessories kit.

White spots on the front panel mark the positions of the soft keys. Touch the utmost left dot on the front panel display with the Dionex menu pen to show the soft key menus:



Fig. 29: Accessing the soft key menus (here: on the autosampler)

# 6 Troubleshooting

The following table provides a summary of possible operating problems, lists probable causes, and suggests remedial actions:

Problem	Probable Cause	Remedial Action
No display on the LCD.	The instrument is not connected to the mains.	Connect the power cord.
	The power is turned off.	Turn on the power.
	The instrument is in standby mode.	Press the Standby key on the instrument's front panel.
	The brightness and/or contrast is not adjusted correctly.	Adjust the brightness and/or contrast ( $\rightarrow$ <b>Contrast</b> and <b>Brightness</b> properties in Chromeleon).
	The fuses blow.	Replace the fuses $(\rightarrow Operating Instructions for the instrument)$
	Replacement fuse blows immediately.	Contact Dionex Service.
	Error in the electronic system	Contact Dionex Service.
The instrument does not work correctly when controlled by Chromeleon.	There is no connection between the instrument and the Chromeleon server PC.	Check the connection to the server PC.
	The USB port on the computer is not ready for operation.	Check the USB port on the computer.
The system has very high backpressure.	One or more capillaries in the system are blocked or damaged by bending.	Check the capillaries in the system, remove the blockage, or replace the capillaries.
The system has very high backpressure at the column and at the pump.	The column is contaminated or blocked.	Rinse or replace the column.
The system has very high backpressure at the pump (the pressure at the column is normal).	The filter frit (porosity: 0.5µm) on the pump's high-pressure side is dirty.	Replace the filter frit.
High baseline drift	The column is contaminated. The system is not sufficiently equilibrated.	Clean or replace the column. Flush the system until equilibration.
	The solvent is degraded.	Use fresh solvent and check the eluent filter frits. In aqueous solvents, growth of microorganisms is possible.
	The environmental conditions are unstable.	Make sure that the temperature and the humidity are constant.
	The detector is not warmed up.	Allow the full detector warm-up time.
	Detector problem	$\rightarrow$ Detector manual

Problem	Probable Cause	Remedial Action
High noise level, pulsation	There are pressure fluctuations from the pump. There are gas bubbles in the system. The solvent is degraded. The detector is defective. The lamp is too old.	Prime the pump; check general function ( $\rightarrow$ <i>Pump manual</i> ). Prime the system ( $\rightarrow$ <i>Pump manual</i> ). Use fresh solvent. Contact Dionex Service. Replace the detector lamp.
Peak broadening, increased dead time	The capillary's inner diameter is too large. The filter frits on the solvent lines are clogged. The capillaries are clogged. The sample loop is clogged. The proportioning valve is defective. The column is overloaded or contaminated. The solvent is degraded. The flow splitter is clogged or defective.	Change the capillary. Check the filter for permeability. Replace the filter frit if necessary ( $\rightarrow$ Pump manual). Replace the capillaries. Replace the sample loop ( $\rightarrow$ Autosampler manual). Contact Dionex Service. Clean or replace the column. Use fresh solvent. Replace the splitter cartridge ( $\rightarrow$ Flow Manager manual). If this does not solve the problem, contact Dionex Service.
Triangular peaks	The column is overloaded (the protein concentration is too high).	Dilute the sample.
Additional peaks appear in the injection peak.	With gradients, the equilibration time after the flush cycle is too short or the dead volume is too high.	Increase the equilibration time and/or eliminate possibly existing dead volume.
Poor reproducibility	The autosampler draws air from the vial. There are gas bubbles in the syringe. There is a gas bubble in the flow path. There is a gas bubble at the bottom of the vial. The autosampler, the injection valve, or the syringe valve is not tight.	There is not enough sample in the vial, the settings for the <b>SampleHeight</b> and/or <b>TransLiquidHeight</b> autosampler parameters are incorrect, or there are too many replicates $(\rightarrow Operating Instructions \text{ for the autosampler})$ . Flush the syringe $(\rightarrow Autosampler manual)$ . Perform a wash cycle $(\rightarrow Autosampler manual)$ . Remove gas bubble from vial. $\rightarrow Autosampler manual$

Problem	Probable Cause	Remedial Action
Poor reproducibility (cont'd)	Carry-over occurs in the system.	Flush the needle using an appropriate solvent.
	The capillary connections are not installed properly or they are not	Check and tighten the capillary connections.
	tight.	Exchange the needle if necessary
		$(\rightarrow Autosampler manual).$
	There are dead volumes in the capillary connections.	Replace the fittings.
	There are dead volumes in the trap column connections.	$\rightarrow$ section 3.7
	The pump head seals are not tight.	Replace the seals ( $\rightarrow Pump$ manual).
	The sample is unstable and decomposes.	Use new sample or change the conditions.
	The gradient is not reproducible.	Change the gradient.
		Check the pump function and degassing.
		Check the suction frits for obstruction and exchange as necessary.
	The environmental conditions are unstable.	Make sure that the temperature and air humidity are constant.
	Contamination occurs	(Use a column thermostat.) Flush the system using an
	somewhere in the system.	appropriate solvent.
No flow	The system is leaking.	Find and eliminate the leak.
	The check valves are not installed properly in the pump (not in direction of flow) or defective.	Correctly install or replace the check values ( $\rightarrow$ <i>Pump manual</i> ).
	There is air in the solvent or in the pump head(s).	Purge the pump ( $\rightarrow Pump$ manual).
	The pump's inline filter is clogged.	Replace the filter ( $\rightarrow Pump$ manual).
Flow fluctuation	The inlet path is blocked.	Check the pump's inlet lines, filter, proportioning valve etc. for signs of blockage.
	There is air in the inlet path.	Purge the pump ( $\rightarrow Pump$ manual) and check the degasser.
	The pump's check valves are dirty or defective.	Clean or replace the check valves if necessary ( $\rightarrow$ <i>Pump manual</i> ).
	The pump head seals are not tight.	Replace the seals ( $\rightarrow Pump$ manual).
Poor degassing	There is a leak in the capillaries or solvent lines or there are loose connections.	Inspect the capillary and solvent tube connections for leakage; tighten loose fitting connections.
	The flow rate is too high.	Reduce the flow rate.
UV signal drift during the gradient	In applications with TFA, the TFA concentration is incorrect. If the signal drifts upward, solvent B contains too much TFA and vice versa.	Increase or reduce the TFA concentration as needed.

Problem	Probable Cause	Remedial Action
After switching from the loading pump flow to the nano flow, there is a drop in the UV signal, and the signal does not return to the initial level fast enough.	There is dead volume in the connection to the trap column.	$\rightarrow$ section 3.7
	The trap column is defective.	Replace the trap column.
No peaks or only few, poorly resolved peaks in the chromatogram	The trap column is too short, the flow rate of the loading pump is too high and/or the loading time is too long. (The three items interact: If the trap column is too short, peak may be lost because they are not retained. If the loading time is too long or if the loading flow is too high, hydrophilic peaks may be lost. This also affects the reproducibility because the proteins are transported a long way into the trap column.)	Consider the three items. Use a longer trap column, reduce the flow rate from the loading pump and/or reduce the loading time.
Reproducible ghost peaks in the chromatogram.	The degasser channels are contaminated.	Rinse the degasser channels $(\rightarrow Solvent Rack manual).$
	The solvents are degraded or dirty or their purity is insufficient.	Use fresh solvents.
	Contamination occurs somewhere in the system.	Flush the system using an appropriate solvent.
The temperature in the flow manager does not change for some time although the temperature set point has not been reached.	The ambient temperature is too high.	Reduce the ambient temperature (e.g., by ventilating the room).
	The ventilation slots on the sides and/or bottom of the instrument are obstructed.	Make sure that the ventilation slots are not obstructed in any way.
	The front panel door is not completely closed.	When closing the door, make sure that the locking mechanism locks the door.
	The capillaries are placed in such a manner that ambient air can enter the column chamber.	Make sure that the capillaries rest flat on the edge of the housing.
	The door seal is damaged.	Contact Dionex Service.

**Please note:** For information about the error messages that may appear in the Chromeleon Audit Trail if an error occurs or if communication between the instrument and Chromeleon cannot be established, refer to the associated sections in the *Operating Instructions* for the instruments.

# 7 Routine Maintenance

The system modules of the UltiMate 3000 system are made of high-quality components and materials to minimize maintenance requirements. The painted surfaces, as well as the display, are relatively resistant to weak acids, alkali, and organic solvents. Nevertheless, immediately wipe up all liquids spilled onto the modules' surface, using lint-free cloth or paper. If surfaces are exposed for longer periods, these liquids can cause damage.

The *Operating Instructions* for the individual instruments provide instructions for shutting down the instrument and describe all maintenance procedures that can be carried out by the user. Dionex personnel should perform any additional servicing, as well as anual inspections to detect contamination, wear, etc. If unexpected problems occur, please contact Dionex Service.

**1** Please note: Before you return any instrument to Dionex for repair, contact Dionex Service or your local distributor. An RMA (Return Material Authorization) number is required in order to track your instrument. Always use the original packaging when shipping the module. Shipping the instrument in anything other than the original packaging will void the warranty. Refer to the warranty statement in the terms of sale for more information.

## 8 Accessories and Spare Parts

Dionex accessories and spare parts are always maintained at the latest technical standard. Therefore, part numbers are subject to alteration. However, updated parts will always be compatible with the parts they replace.

- Please note: The part numbers always refer to the packing unit. Unless stated otherwise, the packing unit is one unit.
- **Please note:** For more information about which accessories and/or spare parts are available for the individual modules, refer to the *Operating Instructions* for the instruments.
- **Please note:** Use original Dionex spare parts only. Substituting non-Dionex parts or using non-Dionex accessories may impair the performance of the system.

## 8.1 Basic Configuration Kits

The kits include all parts that are necessary for the configuration!

#### 8.1.1 Standard Devices

Description	Part No.
Basic Configuration Kit for Nano HPLC	6720.0065
Basic Configuration Kit for Capillary HPLC	6720.0082
Basic Configuration Kit for Micro HPLC	6720.0083
Basic Configuration Kit for monolithic HPLC	6720.0084

#### 8.1.2 Biocompatible Devices

Description	Part No.
Basic Configuration Kit (biocompatible) for Nano HPLC	6721.0065
Basic Configuration Kit (biocompatible) for Capillary HPLC	6721.0082
Basic Configuration Kit (biocompatible) for Micro HPLC	6721.0083
Basic Configuration Kit (biocompatible) for monolithic HPLC	6721.0084

## 8.2 Direct Injection Kits

#### 8.2.1 Standard Devices:

Description	Part No.
Direct Injection Kit for Nano HPLC	6720.0042
Direct Injection Kit for Capillary HPLC,	6720.0043
Direct Injection Kit for Micro HPLC	6720.0046
Direct Injection Kit for monolithic HPLC	6720.0044

#### 8.2.2 Biocompatible Devices:

Description	Part No.
Direct Injection Kit (biocompatible) for Nano HPLC	6721.0042
Direct Injection Kit (biocompatible) for Capillary HPLC	6721.0043
Direct Injection Kit (biocompatible) for Micro HPLC	6721.0046
Direct Injection Kit (biocompatible) for monolithic HPLC	6721.0044

## 8.3 Preconcentration Kits

The kits include all parts that are necessary for the configuration!

#### 8.3.1 Standard Devices:

Description	Part No.
Preconcentration Kit for Nano HPLC	6720.0047
Preconcentration Kit for Capillary HPLC	6720.0048
Preconcentration Kit for Monolithic HPLC	6720.0049

#### 8.3.2 Biocompatible Devices

Description	Part No.
Preconcentration Kit (biocompatible) for Nano HPLC	6721.0047
Preconcentration Kit (biocompatible) for Capillary HPLC	6721.0048
Preconcentration Kit (biocompatible) for Monolithic HPLC	6721.0049

## 8.4 2D LC Salt Plug Kits

The kits include all parts that are necessary for the configuration!

#### 8.4.1 Standard Devices

Description	Part No.
2D LC Salt Plug Kit for Nano HPLC	6720.0051

#### 8.4.2 Biocompatible Devices

Description	Part No.
2D LC Salt Plug Kit (biocompatible) for Nano HPLC, including:	6721.0051

### 8.5 Comprehensive 2D LC Kits

The kits include all parts that are necessary for the configuration!

#### 8.5.1 Standard Devices

Description	Part No.
2D LC Comprehensive Kit for Capillary/Nano HPLC	6720.0053

#### 8.5.2 Biocompatible Devices

Description	Part No.
2D LC Comprehensive Kit (biocompatible) for Capillary/Nano HPLC	6721.0053

## 8.6 Parallel LC Kits

The kits include all parts that are necessary for the configuration!

#### 8.6.1 Standard Devices

Description	Part No.
Parallel LC Kit, Nano/Nano	6720.0054
Parallel LC Kit, Cap/Cap	6720.0055
Parallel LC Kit, 2x Monolithic	6720.0056

#### 8.6.2 Biocompatible Devices

Description	Part No.
Parallel LC Kit (biocompatible), Nano/Nano	6721.0054
Parallel LC Kit (biocompatible), Cap/Cap	6721.0055
Parallel LC Kit (biocompatible), 2x Monolithic	6721.0056

## 8.7 Upgrade Kits

#### 8.7.1 Standard Devices

Description	Part No
Upgrade CAP LC Kit with flow cell for UVD-3000	6720.0094
Upgrade CAP LC Kit without flow cell	6720.0095
Upgrade Micro LC Kit with flow cell for UVD-3000	6720.0096
Upgrade Micro LC Kit without flow cell	6720.0097

#### 8.7.2 Biocompatible Devices

Description	Part No
Upgrade CAP LC Kit with flow cell for UVD-3000 (biocompatibel)	6721.0094
Upgrade CAP LC Kit without flow cell (biocompatibel)	6721.0095
Upgrade Micro LC Kit with flow cell for UVD-3000 (biocompatibel)	6721.0096
Upgrade Micro LC Kit without flow cell (biocompatibel)	6721.0097

# **9** Technical Information

Column flow range:	50 nl/min to 2.5 ml/min using predefined splitter	
	cartridges and active flow control	
Flow control:	Electronically controlled and actively balanced flow splitter, independent from solvent composition and column backpressure	
Gradient delay time:	Typically less than 1.5 min to splitter outlet at 200 nl/min	
Sample thermostatting:	4 to 45°C, max. 22 °C below ambient	
Thermostatted column compartment and switching valves:	5–70 °C (max. 15 °C below ambient)	
UV detection:	Dedicated flow cells for nano-, capillary- and micro HPLC with 10 mm path lengths	
User input and display:	Large LCDs, programmable to show important system parameters Soft keys for operation during installation and maintenance	
System control:	All functions and parameters are software controlled via USB 1.1 connection	
GLP features:	In Chromeleon: Automatic equipment qualification (AutoQ), System Wellness monitoring and recording of all system parameters in audit trail by Chromeleon software	
MS software interfacing:	Via the appropriate add-ons to Chromeleon, single-point control for Analyst <sup>®</sup> (Applied Biosystems/ MDS Sciex) HyStar <sup>™</sup> (Bruker Daltonics) Xcalibur <sup>®</sup> (Thermo Electron Corporation)	

Technical information: May 2006

All technical specifications are subject to change without notice.

For information about the technical specification on the individual system modules, refer to the *Operating Instructions* for the instrument.

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