UHPLC+ focused Solution

Giving you more.

Tandem Operation

Quick Installation Guide

Thermo Scientific Dionex UltiMate 3000
LC, RSLC, and x2 Dual Gradient Systems

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Considerations

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How to use this Manual

These Quick Installation Guides describe how to assemble a dedicated Thermo Scientific Dionex UltiMate 3000 system with Thermo Scientific Dionex UHPLC$^+$ Solution hardware. It is assumed that the individual using this manual has a sufficient training in the use of UltiMate™ 3000 systems, is aware of the potential hazards including (but not limited to) electrical hazards, chemical solvent hazards, exposure to UV radiation, and exposure to pressurized solvents.

The layout of this manual is designed to provide quick reference to the sections of interest. However, a review of the whole manual is recommended to obtain a full understanding of the Dionex UHPLC$^+$ Solution and to operate the modified instrument properly.

This manual is provided as state-of-the-art. Every effort has been made to supply complete and accurate information. All technical specifications and programs have been developed with the utmost care. However, Thermo Fisher Scientific assumes no responsibility and cannot be held liable for any errors, omissions, damage or loss that might result from any use of this manual or the information contained therein. We appreciate your help in eliminating any errors that may appear in this document.

The Tip and Note signs shown below are included in various locations in this manual and provide the following information:

Tip:	**Indicates general information intended to optimize the workflow or indicates additional information.**
Note:	**Indicates specific information intended to run the UHPLC$^+$ Solution properly.**

All UHPLC$^+$ Solution Kits are delivered (besides Viper™ capillaries and other equipment) with a printed Quick Installation Guide and a printed laminated Flow Scheme Card. The kit also contains an appropriate UHPLC$^+$ Solution CD with digital versions of those manuals described above, Operating Instructions, and demo data.

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1. Introduction

Thermo Fisher Scientific has developed a number of Dionex UHPLC$^+$ Solutions to obtain the following benefits:

- Combining the advanced features of UHPLC$^+$ focused UltiMate 3000 hardware, Chromeleon Chromatography Data System software, and Viper UHPLC$^+$ Solutions Kits.
- The best possible synergy of performance, reliability and ease-of-operation of UHPLC$^+$ focused UltiMate 3000 system(s).
- Solving typical analytical challenges, such as method development, sample preparation, or increasing throughput and productivity.

All Viper UHPLC$^+$ Solution Kits are predominantly designed for use with column lengths of up to 250 mm and work for the given chromatographic scenario of Chapter 2, which provides the recommended schematic system setup(s).

1.1 Viper UHPLC$^+$ Solution Kits

All Viper UHPLC$^+$ Solution Kits are delivered in a high-quality box with a foam inlay. Independent of the ordered kit, you will always receive the same box, accommodating all necessary accessories and capillaries for your individual Viper UHPLC$^+$ Solution Kit.

Figure 1: Packaging box for all Viper UHPLC$^+$ Solution Kits

1.2 Typical System Setups

1.2.1 Single-Stack

In many cases, bench space is limited; hence the UHPLC$^+$ focused UltiMate 3000 systems are designed to consume as little space as possible.

Thermo Fisher Scientific assumes that a single-stack setup (figure 2) is the most common case. Therefore, the Viper UHPLC$^+$ Solution Kits have been optimized for this scenario.

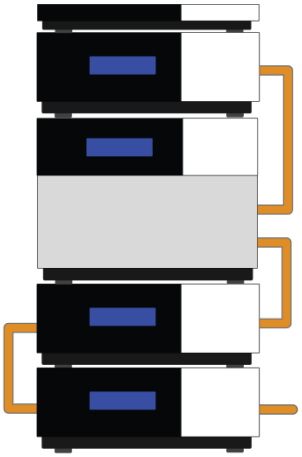

Figure 2: Single-stack

Tip:

Figure 2 illustrates the schematic installation of the flexible Viper capillaries in general. Thermo Fisher Scientific recommends installing all capillaries inside the modules as needed.

1.2.2 Dual-Stack

Whenever bench space is of no concern but reduced system height is more important to users, the system can be configured as a dual-stack. Several different combinations are conceivable, depending on the kind and number of modules included in a system. The Viper kits have been carefully developed to cover as many individual situations as possible for the use of all UHPLC$^+$ focused UltiMate 3000 modules in single-stack or dual-stack configurations. In some cases, however, limitations may occur. Figures 3 and 4 illustrate the two most common dual-stack configurations.

Figure 3: Dual-stack with U-shaped fluidic line

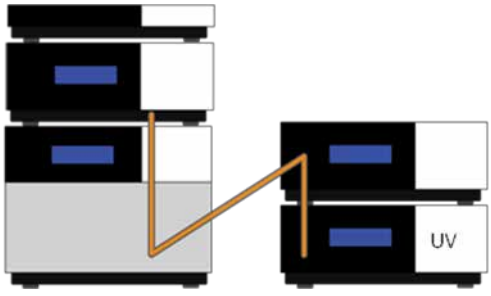

Figure 4: Dual-stack with Z-shaped fluidics

1.3 Connecting the Eluent Line to the Solvent Reservoir

Figure 5: Retaining guide

1. Feed the eluent line through the retaining guide and then into the opening of the reservoir cap.

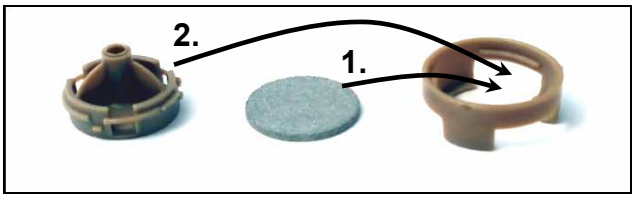

Figure 6: Eluent filter components

2. Assemble the eluent filter (figure 6).

3. Slide the eluent filter assembly onto the end of the eluent line.

4. Verify that the end of the eluent line is cut straight and is not deformed. If necessary, cut the tubing with a sharp knife or blade. Use only the eluent lines shipped with the solvent rack.

5. Place the complete assembly in the solvent reservoir.

6. Make sure that the eluent filter assembly reaches the bottom of the solvent reservoir.

7. Hand-tighten the solvent reservoir caps.

Note: **To exchange the tubing, first remove the frit, and then remove the eluent tube before removing the retaining guide.**

Important: **Always use eluent tubing with filter frits for drawing eluents from the eluent bottles. This prevents contamination of the HPLC system.**

Note: **Regularly check the frits for permeability. Especially when working with aqueous solvents, algae and other microorganisms can grow on the frits causing clogging. Therefore, exchange the solvents at regular intervals. Rinse the bottles thoroughly with fresh eluent before reusing them. Replace the frits when necessary.**

1.4 Viper Capillaries

When working with small column volumes, capillaries and connections require special attention. But even with conventional columns and moderate pressures, mediocre connections can cause retention time shifts and peak distortions. Conventional stainless steel HPLC connectors use a ferrule and a nut to establish connections. A flaw of the conventional design is that a void volume-free connection is not guaranteed, particularly when changing the tubing between differently shaped threads (e.g., when changing a column). Viper connectors provide zero dead volume by sealing at the tubing tip, hence ensuring optimized connections of conventional HPLC and modern UHPLC systems without any additional tools.

Even though Viper withstands UHPLC backpressures of up to 1,200 bar (17,400 psi), it is a finger-tight fitting system, which requires only small torques to seal, and is compatible with third-party valves and unions.

1.5 nanoViper Capillaries

When working with capillary and nano LC, a single bad capillary connection can cause the difference between quality results and a failed experiment. The typical low flow rates in these applications make them exceptionally sensitive to dead volumes. Even when the nut, ferrule, PEEK sleeve, and column have been connected dead-volume free, care must be taken to not over-tighten and damage the fused silica or, worse, the separation column.

Remedying this, nanoViper is a finger-tight connection system for nano LC connections which eliminates the assembly of PEEK sleeve connections. It is preassembled to maximize ease-of-use. The nanoViper fitting is capable of withstanding pressures up to 1,034 bar (14,500 psi), and is compatible with third-party valves and unions.

1.6 Viper Labeling

Each Viper capillary comes with a labeling clip. This clip can be easily detached and reattached to 1/16” and 1/32” OD capillaries. In addition, a set of 5 blank clips and stickers is supplied for individual labeling purposes.

Figure 7: Removable labeling clip with sticker for individual capillary labeling.

1.7 Tightening a Viper

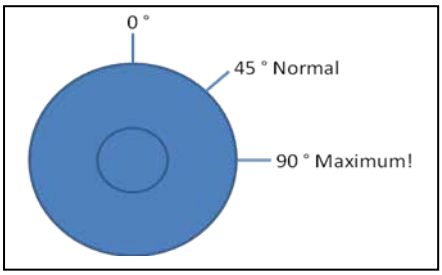

Figure 8: Schematic for tightening a Viper

Attach the Viper to the target thread and tighten the screw slowly until you feel the very first resistance. This is the 0° mark. Do not use tools other than the black knurl for opening and closing a connection. Tighten the screw clockwise to an angle between 0° and 45° and start operating your LC system at the desired working pressure. Verify that all connections seal properly. Usually, the Viper fitting is tight after the first attempt.

If leakage occurs under these conditions, tighten the screw(s) gradually further until the connection seals properly. Do not turn the screw beyond an angle of 90° to avoid damages of the PEEK seal by over-tightening. Do not apply brute force. To extend the life-time of Viper, open and close connections only at atmospheric system pressures. Opening and closing Viper connections at high system pressures may reduce the life-time of the fitting system.

1.8 Eluent Conditioner

Pre-column heating and post-column cooling improve the chromatographic performance, especially when elevated temperatures are used. Pre-column heating brings the solvent to the column temperature before the solvent enters the column. This avoids temperature gradients in the column. Post-column cooling provides additional safety and optimized detection parameters when working at higher column temperatures.

Thermo Fisher Scientific offers Dionex Eluent Pre-Column Heaters that can be placed inside all Dionex TCC-3x00 SD and RS Thermostatted Column Compartments to warm-up the eluent before the column.

Only a Dionex TCC-3x00 RS Thermostatted Column Compartment contains a Post-Column-Cooler device in which a Dionex Eluent Post-Column Cooler can be placed and then cools down the eluent before it enters the detector.

Small internal volumes (2 µL, 7 µL, or 11 µL) ensure minimal additional gradient delay and extra column volumes respectively. The decision which eluent conditioner is appropriate for the respective application depends on various factors, such as the flow rate and the difference between the ambient and column temperature. Typically, a 2 µL eluent conditioner is sufficiently effective, even with challenging application requirements.

Note: **Information about the installation of the eluent conditioner is given in the manual delivered with the pre-column heater, post-column cooler, or with all TCC-3x00 Thermostatted Column Compartments.**

2. Tandem Operation

Standard (P/N 6040.2804)
Rapid Separation (P/N 6040.2803)

2.1 Benefit of Tandem Operation

Tandem Operation is UHPLC$^+$ solution for increasing the throughput of gradient separations between 50% and 100%. With Tandem Operation, two different flow paths are used, allowing for off-line equilibration of one column while a second column is used for the current analysis.

Tandem Operation offers a throughput increase without the need for further method optimization. This eliminates the need for method revalidation and revision of corresponding documentation, which might be necessary with other throughput-increase approaches.

The UHPLC$^+$ focused system with Tandem Operation includes a dual-gradient pump with the ability to perform two ternary gradients at the same time, a thermostatted column compartment with one 2-position 10-port valve for column switching, a split loop well-plate autosampler, and a stackable Dionex detector, all of which are fully controlled by the Chromeleon Chromatography Data System.

2.2 Scope of Delivery

Table 1: Scope of delivery for the Viper Tandem Operation Kit, RS system (P/N 6040.2803) or SD system (P/N 6040.2804)

Description	Quantity
Viper capillary (SST), ID x L 0.13 mm/0.005” or 0.18 mm/0.007” x 150 mm	1 pc.
Viper capillary (SST), ID x L 0.13 mm/0.005” or 0.18 mm/0.007” x 250 mm	2 pcs.
Viper capillary (SST), ID x L 0.13 mm/0.005” or 0.18 mm/0.007” x 350 mm	1 pc.
Viper capillary (SST), ID x L 0.18 mm/0.007” x 450 mm	1 pc.
Viper capillary (SST), ID x L 0.13 mm/0.005” or 0.18 mm/0.007” x 550 mm	3 pcs.
Viper capillary (SST), ID x L 0.18 mm/0.007” x 950 mm	1 pc.
PEEK capillary for valve waste connection, 1/16”, 0.25 mm/0.010” ID	1 m
Viper union for direct connection of two Viper capillaries	2 pcs.
Capillary marker	5 pcs.
Labels for capillary marker	8 pcs.
One-piece finger tight fitting 1/16", PEEK	1 pc.
Eluent pre-heater with Viper fitting, ID 0.13 mm ID, volume 2 µL	1 pc.
Laminated Flow Scheme	1 pc.
Quick Installation Guide	1 pc.
UHPLC$^+$ Solution CD for Tandem Operation	1 pc.

Note: SD system: All Viper Capillaries are delivered with an ID of 0.18 mm/0.007”.

2.3 Stacking the Modules

Please stack the modules as shown below. The picture is a schematic to illustrate the course of the flexible Viper capillaries in general. Thermo Fisher Scientific recommends installing all capillaries inside the modules as needed.

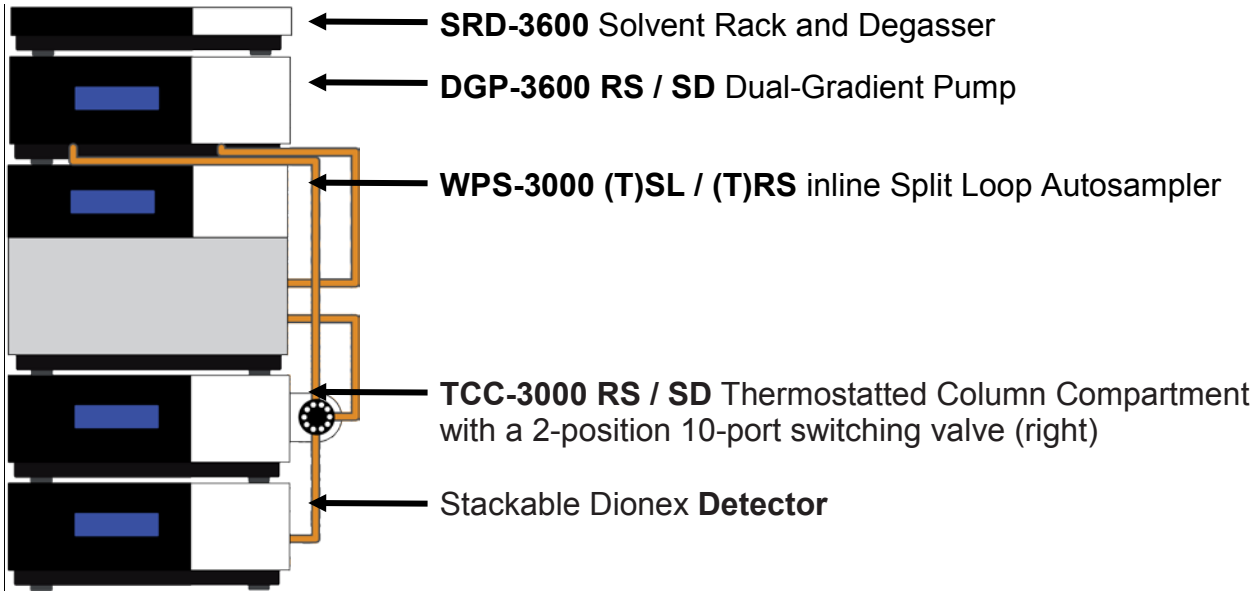

Figure 10: Tandem Operation setup

⚠ **Important:** **All modules are primed with 2-propanol at the factory. Prior to initial operation of the system, make sure that all used solvents are miscible with 2-propanol and equilibrate your system. Otherwise, perform appropriate intermediate flushing steps.**

2.4 Flow Schematics

The figure below shows the schematic setup of the tandem operation configuration. Install your Viper capillaries according to the information of table 2 and the system stack schematic of figure 10.

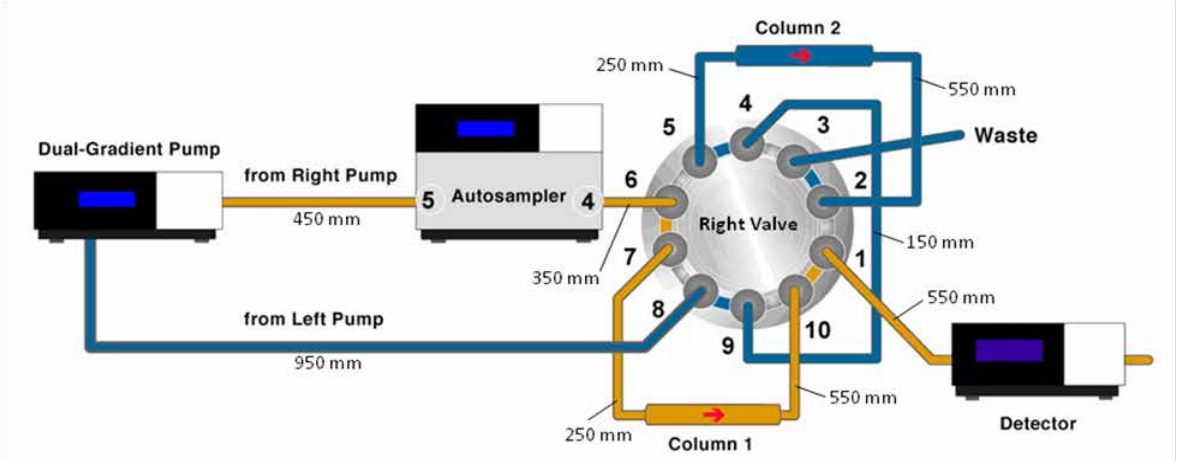

Figure 11: Flow schematic of tandem operation setup

Table 2: Detailed description of capillary connections of the Tandem Operation setup

Description of Connections	Capillary Dimensions (ID x L)
PumpLeft outlet – TCC 10-port valve right, port 8	0.18 mm/0.007” x 950 mm
PumpRight outlet – WPS sampler valve, port 5	0.18 mm/0.007” x 450 mm
TCC 10-port valve right, port 7 – Column 1 inlet	RS: 0.13 mm/0.005” x 250 mm SD: 0.18 mm/0.007” x 250 mm
TCC 10-port valve right, port 5 – Column 2 inlet	RS: 0.13 mm/0.005” x 250 mm SD: 0.18 mm/0.007” x 250 mm
TCC 10-port valve right, port 4 – TCC 10-port valve right, port 9	RS: 0.13 mm/0.005” x 150 mm SD: 0.18 mm/0.007” x 150 mm
TCC 10-port valve right, port 3 – Waste	0.25 mm/0.010” x 1,000 mm (PEEK)
WPS sampler valve, port 4 – TCC 10-port valve right, port 6	RS: 0.13 mm/0.005” x 350 mm SD: 0.18 mm/0.007” x 350 mm
Column 1 outlet – TCC 10-port valve right, port 10	RS: 0.13 mm/0.005” x 550 mm SD: 0.18 mm/0.007” x 550 mm
Column 2 outlet – TCC 10-port valve right, port 2	RS: 0.13 mm/0.005” x 550 mm SD: 0.18 mm/0.007” x 550 mm
TCC 10-port valve right, port 1 – Detector inlet	RS: 0.13 mm/0.005” x 550 mm SD: 0.18 mm/0.007” x 550 mm

Note: **In case of a dual-stack setup, the TCC 10-port valve right turns into a TCC 10-port valve left. Both stacks should have a distance of about 7 cm (2.8 inches). Connecting a post-column cooler with a stackable Dionex detector does not work without additional capillaries when setting up the system in the dual-stack U-configuration.**