# Thermo Scientific Dionex UltiMate 3000 RSLCnano System

Thermo Scientific<sup>™</sup> Dionex<sup>™</sup> UltiMate<sup>™</sup> 3000 products are UHPLC compatible by design, establishing the new standard in conventional LC. Integrating hardware, software, and separation chemistry, we offer UHPLC to everyone, for all needs.



The Thermo Scientific Dionex UltiMate 3000 RSLCnano system provides ultrafast, ultrahigh-resolution separations using a powerful nano LC pump that can deliver up to 800 bar pressure. The thoughtfully engineered system design together with Thermo Scientific Dionex nanoViper<sup>™</sup> fingertight fittings makes this system easy-to-use, allowing operators to set up advanced solutions in minutes.

Thermo Scientific Acclaim<sup>™</sup> PepMap<sup>™</sup> RSLC columns efficiently resolve the most challenging biological samples. This system is ideally suited for coupling to mass spectrometry, both with ESI and MALDI interfaces.

#### System Features

- Continuous direct flow
- Small gradient delay volume of only 25 nL
- Flow delivery from 20 nL/min up to 50 µL/min at a maximum pressure of 800 bar
- Unparalleled gradient precision
- Fully biocompatible pump system
- Integrated ternary gradient pump, 10–2500 µL/min

- Up to two low-dispersion 2-position switching valves
- Nano, capillary, and micro LC applications
- Can perform multidimensional separations
- Easy coupling to ESI and MALDI MS
- UHPLC-compatible fingertight connections
- High-resolution columns
- Zero-sample-loss injection
- High-precision sample injections down to 10 nL
- Sample fractionation and automated reinjection
- Sample cooling down to 4 °C
- UV detector for nano LC using a 3 nL flow cell
- Data collection rate of 200 Hz
- Automatic IQ/OQ/PQ through Thermo Scientific Dionex Chromeleon<sup>™</sup> software



#### **NCS-3500RS Module**

The Thermo Scientific Dionex NCS-3500RS module integrates a continuous direct flow HPG nano pump, a ternary LPG micro pump, and a heated column compartment with up to two UHPLC-compatible switching valves. It is designed for maximum flexibility and ease-of-use, supporting any workflow in nano, capillary, and micro LC. Optimized system geometry and a revolutionary nanoViper fingertight fitting assure optimum LC performance without concerns about connections.

# **HPG Nano Pump**

The continuous direct flow Thermo Scientific Dionex HPG nano pump delivers flow rates from 20 nL/min up to 50  $\mu$ L/min at standard or ultrahigh backpressures up to 800 bar. The pump is designed to deliver gradients at the lowest imaginable flow rates with the exceptionally high precision required for high confidence compound identification.

# **HPG Nano Pump Features**

- Column pressures up to 800 bar (11,600 psi) over the entire flow range from 20 nL/min up to 50 µL/min
- Fast separations with a gradient delay volume as small as 25 nL
- Unparalleled small retention time variations ensured by precise gradient formation and high pressure mixing
- Easily exchangeable flow selector for maximum pump performance and system flexibility

# **LPG Micro Pump Features**

- Ternary gradient
- Flow rates from 10 µL/min up to 2.5 mL/min
- Ideal for on-line sample loading and multidimensional LC workflows

#### NCP-3200RS Module

The HPG nano pump is also available as a stand alone module, the Thermo Scientific Dionex NCP-3200-RS. The specifications are similar to the NCS-3500RS HPG pump. The pump can be used for direct sample analysis or, in combination with the NCS-3500RS module, for advanced applications such as tandem nano LC or 2D-LC.



Figure 1. The NCS-3500RS module supports ultrahigh backpressures up to 800 bar across nano, capillary, and micro LC flow rates. The integrated switching valve(s) together with nanoViper fittings facilitate configuration of any workflow.



Figure 2. High-resolution separation of a complex tryptic digest sample usin a 50 cm 75 μm Acclaim PepMap column. The peak capacity for this separation was 450.



Figure 3. Schematic of the HPG nano pump. The dual piston design for each solvent channel, optimized fluidics, and smart algorithms enable stable flow rates from 20 nL/min up to 50  $\mu$ L/min, independent from backpressure changes caused by ultrafast gradients or a partially blocked capillary like an ESI spray needle.



Figure 4. Unparalleled retention time precision in a nano LC preconcentration analysis of tryptic peptides. Zoom in of overlay of eight consecutive runs with retention time precision  $\leq$  0.05% RSD for all peptides.

| Key Pump Specifications  |  |  |  |  |
|--|--|--|--|--|
| Flow Rate Ranges:  | Solvent Degassing:   |  |  |  |
| HPG Nano Pump: 20 nL/min–50 µL/min                                   | External (optional)  |  |  |  |
| (using dedicated flow selectors)                                     | Wetted Parts:  |  |  |  |
| Flow Selectors (recommended):  | HPG Nano Pump: titanium, PEEK <sup>®</sup> , UHMW-PE, PTFE, FEP, ruby,       |  |  |  |
| Nano: 50 nL/min–1000 nL/min  | sapphire, Al <sub>2</sub> O <sub>3</sub> , fused silica                      |  |  |  |
| Capillary: 500 nL/min–10 µL/min                                      | LPG Micro Pump: titanium, PEEK, UHMW-PE, PTFE, FEP, ruby,                    |  |  |  |
| Micro: 2.5 µL/min–50 µL/min  | sappnire, $2rU_2$ , $Al_2U_3$ , Kairez                                       |  |  |  |
| Custom flow selectors (e.g. for 20 nl/min) are                       | Dimensions ( $n \times w \times d$ ):  |  |  |  |
| available on request.  | NCS-3500RS: $36 \times 42 \times 51$ cm (14.1 $\times$ 16.5 $\times$ 20 in.) |  |  |  |
| LPG Micro Pump: 10–2500 µL/min (gradient from 50 µl/min)             | NCP-3200RS: $21 \times 42 \times 51$ cm (8.3 × 16.5 × 20 in.)                |  |  |  |
| Pressure Range:  | Weight:  |  |  |  |
| 20–800 bar (300–11,600 psi), HPG Nano Pump                           | NCS-3500RS: 32 kg (70.6 lb)  |  |  |  |
| 20–500 bar (300–7250 psi), LPG Micro Pump                            | NCP-3200RS: 17.5 kg (38.6 lb)  |  |  |  |
| Number of Solvent Channels:  | Power Requirements:  |  |  |  |
| HPG Nano Pump: 2   | 100–120 V, 60 Hz   |  |  |  |
| LPG Micro Pump: 3  | 200–240 V, 50 Hz; max 300 VA   |  |  |  |
| Flow Calibration:  | PC Connection:   |  |  |  |
| Semi-automated   | USB 2.0; USB hub with three integrated sockets                               |  |  |  |
| Proportioning Accuracy:  | I/O Interfaces:  |  |  |  |
| <1% of full scale  | Two digital inputs and two programmable outputs                              |  |  |  |
| Proportioning Precision:   | Additional Communication Port:   |  |  |  |
| Typically <0.2% SD   | 15-pin D-Sub port for connection of a solvent rack or degasser               |  |  |  |
| Retention Time RSD in Gradient Mode at 300 nL/min:                   | GLP Features:  |  |  |  |
| <0.2% RSD or <0.1 min SD, whichever is greater                       | System wellness monitoring, column tracking                                  |  |  |  |
| Gradient Delay Volume:   | Safety Features:   |  |  |  |
| <25 nL (pump) and <350 nL (system in preconcentration configuration) | Leak sensor, active rear-seal wash system, excess pressure monitoring        |  |  |  |
| Eluent Bottles:  |  |  |  |  |
| 2 × 100 mL   |  |  |  |  |
| 3 × 500 mL   |  |  |  |  |

# **Column Compartment**

The column compartment has been designed to provide maximum operational flexibility and convenience. Two lowdispersion switching valves and a maximum temperature of 75 °C provide full flexibility for any column-switching experiment. The 10-port or 6-port switching valves can be pulled forward and taken out for easy access and column installation.

#### **Column Compartment Features**

- Thermostatted column compartment from 10 °C above room temperature (RT) to 75 °C
- Up to two low-dispersion 2-position, 10-port or 6-port snap-in valves
- Column identification system for easy data storage



Figure 5. The snap-in switching valve and the proprietary fingertight nanoViper fittings provide the highest level of convenience and confidence for column installation.



Figure 6. Advanced workflows such as phosphopeptide analysis are easily supported by the UltiMate 3000 RSLCnano system using the 2-position switching valves in the thermostated column compartment.

# **Key Column Compartment Specifications**

#### **Temperature Range:** RT + 10 °C-75 °C

# Temperature Accuracy: $\pm 0.5$ °C

**Temperature Stability:**  $\pm$  0.1 °C (at 50 °C setpoint)

Heat-Up Time: From 35 °C to 65 °C in 15 min Switching Valves: Up to two 10-port, 2-position low-dispersion valves Port-to-port volume: 124 nL Maximum pressure: 900 bar (13,050 psi)

Column Capacity: Up to 3 columns Up to 100 cm length (75 µm i.d., coiled)

Safety Features: Humidity sensor, leak sensor

#### WPS-3000TPL RS Autosampler

The Thermo Scientific Dionex WPS-3000TPL RS autosampler uses a 2.4  $\mu$ L needle to allow injection of the smallest sample volumes with high precision and with no sample loss. The injection valve supports injections at ultrahigh pressures, both directly onto the column or onto a sample trapping column. The optional sample fractionation provides the highest application flexibility in multidimensional workflows. The sample compartment is entirely closed to ensure sample stability.

#### **Autosampler Features**

- Microliter pick-up injections for zero sample loss
- Automated wash routines to prevent sample carryover
- Injection valve pressure rating of 900 bar
- User-defined programs for unlimited sample handling capabilities
- Sample thermostating from 4 °C to 45 °C, at least 22 °C below ambient, to help prevent sample degradation
- Dual-needle injection design, supporting injection from sealed sample carriers



Figure 7. Automated sample handling and LC workflows facilitated by the micro fractionation option of the WPS-3000TPL RS autosampler.

- Large injection volume range, from 10 nL up to 125 µL
- Microfractionation option for highest application flexibility
- · Three well plate sample capacity
- Flexible multiple-tray carrier supporting all common sample carriers

# **Key Autosampler Specifications**

Injection Volume Range:

10 nL-125 µL (with installed options)

#### Sample Formats:

96 (deep) well plate, 384 (deep) well plate, 24 deep well plate, sealed or open; 40 standard autosampler vials, 1.8 mL, sealed or open

#### Sample Capacity:

 $3\times$  well plate (128  $\times$  86 mm) 15 $\times$  10 mL vials for reagents, diluents, and transport liquids

#### Injection Cycle Time:

30 s for a 1  $\mu L$  full loop injection

# Injection Methods:

Full loop, partial loop, low-dispersion injection Microliter pick-up, user-defined injection programs

Injection Technique: Needle-in-needle with programmable needle wash

**Injection Vlave Precision:** <0.4% RSD for 1 µL full loop injection

#### Injection Linearity:

Corr. coeff. > 0.9995 from 100-500 nL injections

Carryover: <0.02% with needle wash (caffeine)

**Optional Sample Cooling:** 4 °C-45 °C, at least 22 °C below ambient

Fraction Collection: Microfraction collection option [up to 345 bar (5000 psi)]

Wetted Parts: PEEK, Stainless steel, PCTFE, fused silica

Dimensions (h x w x d):  $36 \times 42 \times 51$  cm ( $16 \times 16.5 \times 20$  in.)

Weight: 22.7 kg (50 lb) including cooling

Power Requirements: 85–260 V, 50/60 Hz, max. 320 W

PC Connection: USB; USB hub with three integrated sockets

**I/O Interfaces:** Four digital inputs and four programmable outputs

# VWD-3400RS UV Detector

The powerful Thermo Scientific Dionex VWD-3400RS UV detector, with its uniquely designed flow cells, allows detection of the smallest amounts of analytes. The use of UV detection provides an ideal tool to monitor low flow LC-MS systems and does not contribute to extracolumn dispersion typically seen when applying too-large flow cells.

#### **UV Detector Features**

- High-sensitivity UV data using dedicated nano and capillary flow cells
- Nano LC flow cell with a volume of 3 nL
- High data collection rate up to 200 Hz
- Up to four wavelengths detected simultaneously



Figure 8. Effect of flow cell volume on resolution of peptides, separated on a 0.2 mm i.d. Thermo Scientific PS-DVB monolithic column at  $2.5 \,\mu$ L/min.



Figure 9. Dedicated UV flow cells for nano and capillary LC.

# **Key Detector Specifications**

# Data Collection Rate:

Up to 200 Hz (in single wavelength mode)

Maximum Number of Channels: 4

# Drift:

4.0 mAU/h

Wavelength Range: 190–900 nm

**Noise:** Typically <0.05 mAU at 254 nm

Lamp: Deuterium lamp, Tungsten lamp

Flow Cell Volume: 3 nL for nano LC 45 nL for capillary LC 180 nL for micro LC Dimensions (h x w x d):  $16 \times 42 \times 51$  cm (6.3 × 16.5 × 20 in.)

Weight: 15 kg (33 lb)

Power Requirements: 85–260 V, 50/60 Hz, max. 150 W

PC Connection: USB

**I/O Interfaces:** Four digital inputs and four digital outputs Two analog inputs (optional DAC module)

# Acclaim PepMap RSLC Columns

The system is complemented by Acclaim PepMap RSLC 2 µm columns, to provide high-efficiency separations with exceptionally high resolution.

# **Column Features**

- Highest resolution in peptide mapping
- nanoViper fittings for easy, tool-free installation
- High sample loadability
- Designed for TFA-free LC-MS, minimizing ion-suppression effects
- Ideally suited for coupling to ESI-MS and MALDI-MS
- Highest column-to-column reproducibility
- Easy-to-use, cutting-edge miniaturized HPLC

# Software

The UltiMate 3000 RSLCnano system is supported by Chromeleon chromatography data management system for convenient system control. Thermo Scientific Dionex DCMS<sup>Link</sup><sup>™</sup> software plug-ins provides single-point control for all major MS platforms.

# **Chromeleon Software Features**

- Intuitive panels for easy system control
- Easy to use diagnostics tests allow users to monitor system performance

# **Dionex DCMS**<sup>Link</sup> Features

Single-point LC-MS control for the following software programs:

- Applied Biosystem Analyst<sup>™</sup>
- Bruker Daltonic Hystar<sup>™</sup>
- Thermo Scientific Xcalibur

# nanoViper Connectivity

Nano LC connections that never fail—this is exactly what nanoViper fittings offer and bring peace of mind to novice, as well as the most experienced, users.

nanoViper fittings are fingertight, UHPLC compatible, and universally applicable to all common 1/16" hardware.

The fittings come integrated on nano columns; a wide range of connection tubing are available. Please refer to the nanoViper tubing matrix for the part number of the tubing you need.



Figure 10. Separation of a cytochrome c digest on an Acclaim PepMap RSLC C18, 2 µm nano column. The high resolution obtained by using a small particle size is immediately clear from the chromatogram.

Peak width at half height for tryptic peptides separated on an Acclaim PepMap RSLC column using a 1.25% ACN min gradient at a flow rate of 300 nL/min.



Figure 11. nanoViper fittings allow you to configure your nano LC application completely tool free.

nanoViper Tubing Matrix with part numbers.

| Longth | ID (µm) Colour Code |             |             |            |               |
|--------|---------------------|-------------|-------------|------------|---------------|
| (mm)   | 20<br>Orange        | 50<br>Brown | 75<br>Black | 100<br>Red | 150<br>Purple |
| 70     | 6041.5120           | 6041.5123   | 6041.5126   | 6041.5810  | 6041.5817     |
| 150    | 6041.5121           | 6041.5124   | 6041.5127   | 6041.5811  | 6041.5818     |
| 250    | -                   | -           | 6041.5730   | 6041.5812  | 6041.5819     |
| 350    | 6041.5240           | 6041.5540   | 6041.5735   | 6041.5813  | 6041.5820     |
| 450    | -                   | -           | -           | 6041.5814  | 6041.5821     |
| 550    | 6041.5260           | 6041.5560   | 6041.5760   | 6041.5815  | 6041.5822     |
| 650    | 6041.5275           | 6041.5575   | 6041.5775   | -          | -             |
| 750    | 6041.5280           | 6041.5580   | 6041.5780   | 6041.5816  | 6041.5823     |
| 950    | 6041.5122           | 6041.5125   | 6041.5128   | -          | -             |

| To order, use the following part numbers and contact your local Thermo Scientific office or distributor nearest you.<br>In the U.S., call (800) 346-6390. In other regions, refer to the phone numbers below. |                                |  |  |  |
|---|--------------------------------|--|--|--|
| Product Description   | Part Number                    |  |  |  |
| Modules   |                                |  |  |  |
| NCS-3500RS Nano LC Pump with Column Compartment   | 5041.0010                      |  |  |  |
| NCS-3500RS Capillary LC Pump with Column Compartment  | 1 Column Compartment 5041.0020 |  |  |  |
| NCP-3200RS Nano/Capillary Pump  | 5041.0030                      |  |  |  |
| WPS-3000TPL RS Pulled-Loop Well Plate Sampler   | ampler 5826.0020               |  |  |  |
| WPS-3000TBPL Biocompatible Pulled-Loop Well Plate Sampler   | 5821.0020                      |  |  |  |
| VWD-3400RS Variable Wavelength Detector   | 5074.0010                      |  |  |  |
| -3400 Solvent Rack with Four Degasser Channels 5035.9245  |                                |  |  |  |
| Accessories   |                                |  |  |  |
| Flow Selector for NCS-3500RS or NCP-3200RS, Nano LC (50-1000 nL/min)  | 6041.0002                      |  |  |  |
| Flow Selector for NCS-3500RS or NCP-3200RS, Capillary LC (0.5–10 µL/min)  | 6041.0003                      |  |  |  |
| Flow Selector for NCS-3500RS or NCP-3200RS, Mirco LC (5-50 µL/min)  | 6041.0014                      |  |  |  |
| Low-Dispersion 2-Position 10-Port Valve for the NCS-3500RS  | 6041.0001                      |  |  |  |
| Low-Dispersion 2-Position 6-Port Valve for the NCS-3500RS   | 6041.0004                      |  |  |  |
| Low-Dispersion 2-Position 10-Port Valve for the NCS-3500RS, Biocompatible, PAEK   | 6041.0012                      |  |  |  |
| UV Flow Cell for Nano LC, 3 nL, for VWD-3400RS  | 6074.0270                      |  |  |  |
| UV Flow Cell for Capillary LC, 45 nL, for VWD-3400RS 6074.0280  |                                |  |  |  |
| V Flow Cell for Micro LC, 180 nL, for VWD-3400RS 6074.0290  |                                |  |  |  |
| Mixer Kit 8 $\mu$ L, for the NCS-3500RS or NCP-3200RS, Capillary  | 6041.7130                      |  |  |  |
| Viper union   | 6040.2304                      |  |  |  |
| RSLCnano Application Kit Overview   |                                |  |  |  |
| Direct Injection nano LC Kit  | 6720.0300                      |  |  |  |
| Direct Injection capillary LC Kit   | 6720.0305                      |  |  |  |
| Preconcentration nano LC Kit  | 6720.0310                      |  |  |  |
| reconcentration capillary LC Kit 6720.0315  |                                |  |  |  |
| 2D salt plugs Kit   | 6720.0325                      |  |  |  |
| Automated off line SCX-RP peptides Kit  | 6720.0330                      |  |  |  |
| Automated off line RP-RP peptides Kit   | 6720.0340                      |  |  |  |
| MS Connection Kit   | 6720.0345                      |  |  |  |
| EASY-Spray Conneciton Kit   | 6720.0395                      |  |  |  |

**Ordering Information** 

#### www.thermoscientific.com/RSLCnano

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