Systems

UltiMate 3000 RSLCnano LC System





Dionex products are UHPLC compatible by design, establishing the new standard in conventional LC. Integrating hardware, software and separation chemistry, Dionex offers UHPLC to everyone—for all needs.

The UltiMate® 3000 RSLCnano system provides ultrafast, ultrahigh-resolution separations using a powerful nano LC pump, able to deliver up to 800 bar pressure. The thoughtfully engineered system design together with nanoViper™ fingertight column fittings makes it an easy-to-use nano LC system, allowing operators to set up advanced solutions in minutes.

Acclaim® PepMap™ RSLC columns efficiently resolve the most challenging biological samples. The UltiMate 3000 RSLCnano 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
- Integrated ternary gradient pump, 10–2500 µL/min
- Up to two low-dispersion 2-position switching valves
- Nano, capillary, and micro LC applications
- 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 Chromeleon® software



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NCS-3500RS Module

The 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 new nanoViper fingertight fitting assure maximum LC performance without concerns about connections.

HPG Nano Pump

The continuous direct flow HPG nano pump delivers flow rates from 20 nL/min up to 50 μ L/min at standard or ultrahigh back pressures 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 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 of up to 800 bar across nano, capillary, and micro LC flow rates. The integrated switching valve(s) together with nanoViper fittings facilitate the set-up of any workflow.

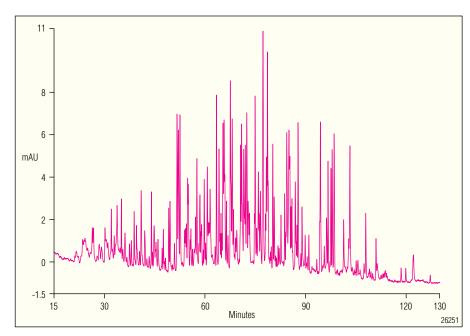


Figure 2. High-resolution separation of a complex tryptic digest sample on a 50 cm long 75 µm Acclaim PepMap column. The peak capacity for this separation was 450.

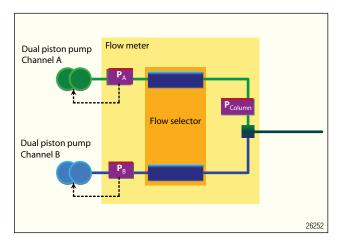


Figure 3. Schematic of the HPG nano pump. The dual piston design for each solvent channel, optimized fluidics, and smart algorithms enable stable flow rates from 20 nL/min up to 50 µL/min, independent from backpressure changes caused by ultrafast gradients or a partially blocked capillary.

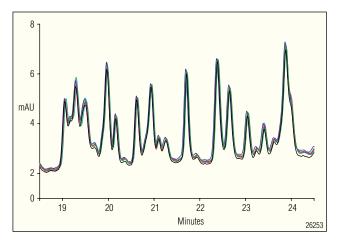


Figure 4. Unparalleled retention time precision in a nano LC preconcentration analysis of tryptic peptides. Zoom in of overlay of eight consecutive runs with retention time precision $\leq 0.05\%$ RSD for all peptides.

KEY PUMP SPECIFICATIONS

Flow Rate Ranges:

HPG Nano Pump: 20 nL/min–50 μL/min (using dedicated flow selectors)

Flow Selectors (recommended [settable]):

Nano: 50 nL/min-1000 nL/min [1 nL/min-1500 nL/min]

Capillary: 500 nL/min-10 µL/min

[1 nL/min–15 µL/min]

Micro: $2.5 \mu L/min-50 \mu L/min$ [1 nL/min-50 $\mu L/min$]

Custom flow selectors are available on request.

LPG Micro Pump: 10–2500 µL/min (NCS-3500RS only)

Pressure Range:

20-800 bar (300-11,600 psi)

20-500 bar (300-7250 psi), loading pump

Number of Solvent Channels:

HPG Nano Pump: 2

LPG Micro Pump: 3

Flow Calibration:

Semi-automated

Proportioning Accuracy:

<1% of full scale

Proportioning Precision:

Typically <0.2% SD

Retention Time RSD in Gradient Mode at 300 nL/min: <0.2% RSD or <0.1 min SD, whichever is greater

Gradient Delay Volume:

<25 nL (pump) and

<350 nL (system in preconcentration configuration)

Eluent Bottles:

 $2 \times 100 \text{ mL}$

 $3 \times 500 \text{ mL}$

Solvent Degassing:

External (optional)

Wetted Parts:

Binary Nano Pump: SST, titanium, PEEK, UHMW-PE, PTFE, FEP, ruby, sapphire, ZrO₂, Al₂O₃, fused silica Ternary Loading Pump:ST, titanium, PEEK, UHMW-PE,

PTFE, FEP, ruby, sapphire, ZrO₂, Al₂O₃, Kalrez[®]

Dimensions $(h \times w \times d)$:

NCS-3500RS: $36 \times 42 \times 51$ cm $(14.1 \times 16.5 \times 20 \text{ in.})$

NCP-3200RS: $21 \times 42 \times 51$ cm $(8.3 \times 16.5 \times 20 \text{ in.})$

Weight:

NCS-3500RS: 32 kg (70.6 lb)

NCP-3200RS: 17.5 kg (38.6 lb)

Power Requirements:

100-120 V, 60 Hz

200-240 V, 50 Hz; max 300 VA

PC Connection:

USB 2.0; USB hub with three integrated sockets

I/O Interfaces:

Two digital inputs and two programmable outputs

Additional Communication Port:

15-pin D-Sub port for connection of a solvent rack or degasser

GLP Features:

System wellness monitoring, column tracking

Safety Features:

Leak sensor, active rear-seal wash system,

excess pressure monitoring

Column Compartment

The column compartment has been designed to provide maximum operational flexibility and convenience. Two low-dispersion 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.

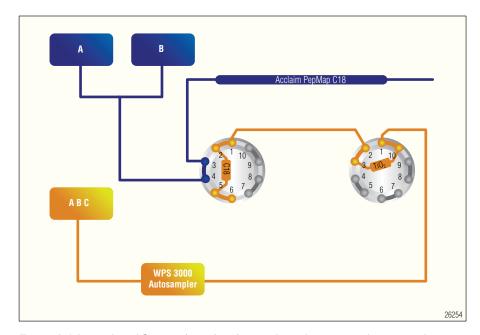


Figure 6. Advanced workflows such as phosphopeptide analysis are easily supported by the UltiMate 3000 RSLCnano system using the 2-position switching valves in the thermostated column compartment.

KEY COLUMN COMPARTMENT SPECIFICATIONS

Temperature Range:

 $RT + 10 \, ^{\circ}C - 75 \, ^{\circ}C$

Temperature Accuracy:

 ± 0.5 °C

Temperature Stability:

 ± 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 WPS-3000TPL RS autosampler uses a 2.4 μ L needle to allow injection of the smallest sample volumes with high precision and without any 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

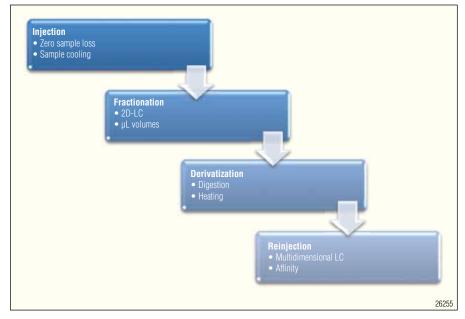


Figure 7. Automated sample handling and LC workflows facilitated by the micro fractionation option of the WPS-3000TPL RS autosampler.

- Large injection volume range, from 10 nL up to 125 μL
- Micro fractionation 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)

 $15 \times 10 \text{ mL}$ vials for reagents, diluents, and transport liquids

Injection Cycle Time:

30 s for a 1 µL 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 Valve 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:

Micro fraction collection option [up to 345 bar (5000 psi)]

Wetted Parts:

PEEK, Stainless steel, PCTFE, fused silica

Dimensions $(h \times w \times d)$:

 $36 \times 42 \times 51$ cm $(16 \times 16.5 \times 20 \text{ in.})$

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 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.

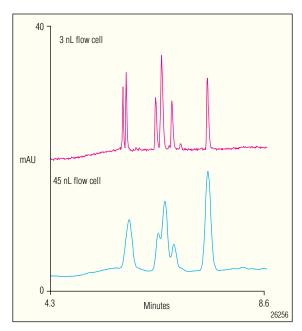


Figure 8. Effect of flow cell volume on resolution of peptides, separated on a 0.2 mm i.d. PS-DVB monolithic column at $2.5~\mu$ L/min.



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:

Four

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 $(h \times w \times d)$:

 $16 \times 42 \times 51 \text{ cm} (6.3 \times 16.5 \times 20 \text{ in.})$

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 a new set of 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. DCMS^{Link™} 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

DCMSLink Features

Single-point LC-MS control for:

- Applied Biosystems' Analyst®
- Bruker Daltonics' Hystar[™]
- Thermo Fisher's Xcalibur®

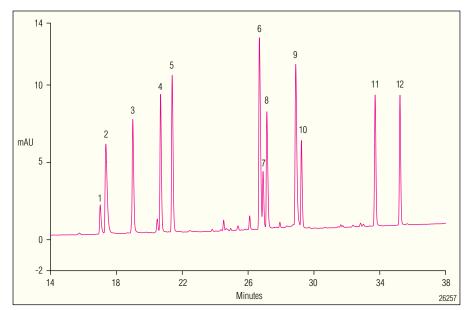


Figure 10. Separation of a cytochrome c digest on an Acclaim PepMap RSLC C18, $2 \mu m$ nano column. The high resolution obtained by using a small particle size is immediately clear from the chromatogram.

Peak	PWHHs
3	5.8
4	5.5
5	5.8
11	5.5
12	5.0

Peak width at half height for tryptic peptides separated on an Acclaim PepMap RSLC column using a 1.25% CH₃CN/min gradient at a flow rate of 300 nL/min.



Figure 11. DCMS^{Link} seamlessly integrates the power of Chromeleon software into the MS software to implement single-point LC-MS control.

ORDERING INFORMATION

To order, use the following part numbers and contact your local Dionex office or distributor nearest you. In the U.S., call (800) 346-6390. In other regions, refer to the phone numbers below.

Product Description	Part Number
NCS-3500RS Nano LC Pump with Column Compartment	5041.0010
NCS-3500RS Capillary LC Pump with Column Compartment	
NCP-3200RS Nano/Capillary Pump	
WPS-3000TPL RS Pulled-Loop Well Plate Sampler	
WPS-3000TBPL Biocompatible Pulled-Loop Well Plate Sampler	
VWD-3400RS Variable Wavelength Detector	
SRD-3400 Solvent Rack with Four Degasser Channels	
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
Flow Meter for the NCS-3500RS or NCP-3200RS, Nano	6041.7901
Flow Meter for the NCS-3500RS or NCP-3200RS, Capillary	6041.7902
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
Mixer Kit 8 μL, for the NCS-3500RS or NCP-3200RS, Capillary	6041.7130

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