



data sheet

### NanoLC-Ultra™ system

The new NanoLC-Ultra is Eksigent's third generation system, delivering superior gradient precision at pressures up to 10,000 psi.

#### Introducing the NanoLC-Ultra family of high pressure HPLCs for proteomics research

The higher pressure capability of the NanoLC-Ultra platform allows the use of longer columns and/or columns packed with smaller particles for higher resolution separations of complex (digested) protein samples. Moreover, the NanoLC-Ultra family features self-priming and self-purging pumps for easier changing of solvents, and a temperature-controlled column compartment for maintaining exceptional retention time reproducibility.

Eksigent's proven Microfluidic Flow Control™ (MFC) technology guarantees reliable and reproducible results, while integration with major mass spectrometer software packages makes the system a seamless addition to almost any nanoLC-MS workflow.

A range of different NanoLC-Ultra system configurations are available for various proteomics applications—from single gradient systems for direct sample injection, to dual gradient systems for multidimensional separations, and dual column set-up for increased throughput.



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The next generation NanoLC system from Eksigent promises to deliver high resolution separations, combined with excellent reproducibility, resulting in improved identification of low abundance peptides/proteins with nanoLC-MS.  
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## Key advantages of the new NanoLC-Ultra

The NanoLC-Ultra system is a proteomics lab workhorse, delivering stable, reproducible gradient separations for peptide/protein identification, phosphopeptide analysis, biomarker discovery, metabolomics and more.

### Splitless, nano-scale flow control

Eksigent's Microfluidic Flow Control (MFC) technology (see Figure 1) uses fast flow measurement in combination with automatic feedback to a regulated pressure source to generate accurate and reproducible flow rates with a resolution better than 1 nL/min. By combining true microfluidic pump control with direct feedback of flow rate, the system eliminates the flow inaccuracies and plumbing problems caused by splitter-based systems. The result is extremely reproducible gradients down to 50 nL/min, without the use of flow splitting, and observed gradient reproducibility typically better than 0.5% RSD (see Figure 2). And the self-priming/self-purging design makes the NanoLC-Ultra pump even easier to set-up and run.

### 10,000 psi maximum pressure

Ultra high pressure LC has been successfully applied in small molecule analyses using 2.1 mm ID columns for increased separation efficiencies and/or analysis speed. For the identification of proteins using LC-MS in proteomics, NanoLC is required because of the increased sensitivity obtained by nanospray mass spectrometry.

The operating pressure of 10,000 psi obtained with the NanoLC-Ultra system enables the use of longer columns, or columns packed with smaller particles, for the analysis of increasingly complex proteomics samples (see Figure 3).

### Retention time reproducibility

Maintaining a constant column temperature is requisite to obtaining the best possible retention time reproducibility. To address this requirement, NanoLC-Ultra systems are equipped with a temperature-controlled column compartment with integrated 10-port valves, that can be regulated to a temperature up to 40°C.

Elevated temperatures also can be utilized to decrease column pressure and/or improve separation efficiency. For advanced applications like sample pre-concentration or dual-column set-up, the NanoLC-Ultra system offers up to two 10-port nano valves rated at 10,000 psi that are integrated into the column compartment. These 10-port valves have an extremely low port-to-port volume of <25 nL, along with a proprietary coating that increases the lifetime of the valve and reduces sample loss.

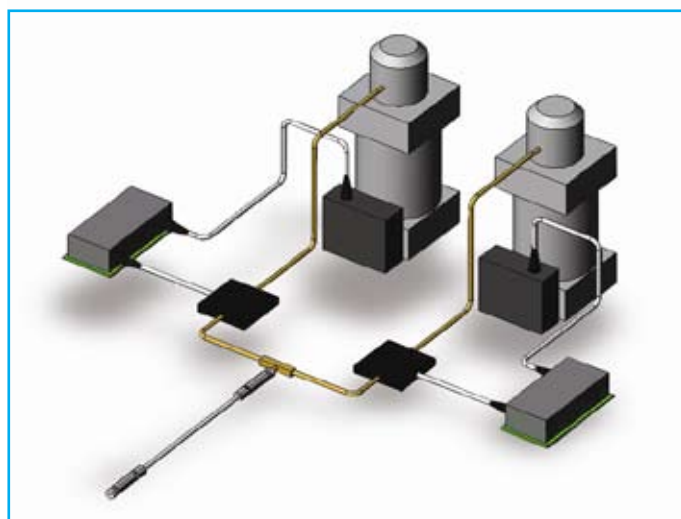


Figure 1. Microfluidic Flow Control

With MFC technology, direct pumping is used to deliver accurate, repeatable gradients. Each pressure source delivers fluid with an actual flow rate monitored using the flow modules in each channel. A CPU closes the loop by making any necessary adjustments to the flow rate using electro-pneumatic controllers.

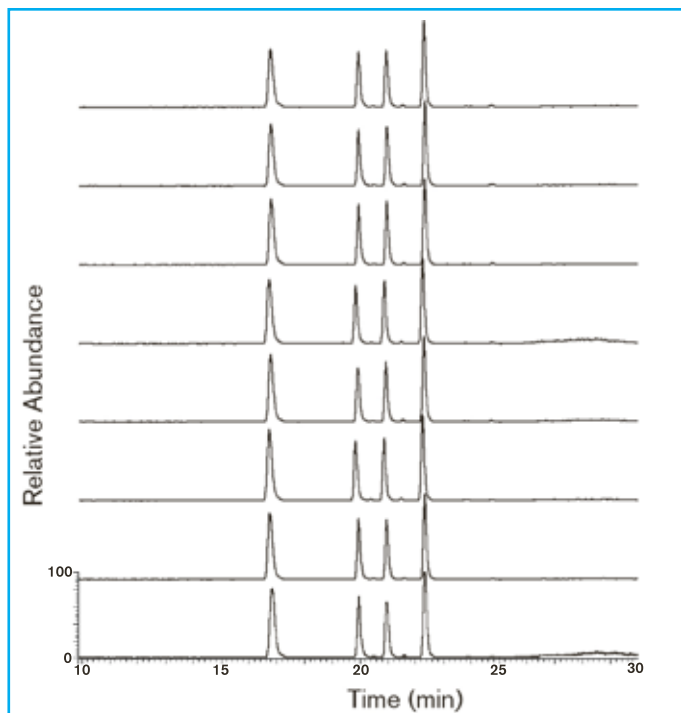


Figure 2. Retention time reproducibility of the NanoLC-Ultra System

This figure shows eight consecutive separations of a peptide test mix on a 75  $\mu\text{m}$  ID x 15 cm C18 NanoLC column. Back pressure was 9,000 psi. The RSD's on retention time are less than .3% for all components.

## Peak parking

Because of the excellent control over the flow rate offered by our MFC technology, the NanoLC-Ultra systems can operate in a “peak parking mode” where the flow rate is temporarily decreased to facilitate longer MS interrogation of an analyte of interest. The result is extended MS/MS analysis acquisition time that improves the MS identification of co-eluting and low abundance peptides.

## Mass spec software integration

With Eksigent's Control Software, users can rapidly set-up experiments and create or modify methods. Specialized diagnostic tools make the system easy to learn, maintain and operate. The software continuously monitors pressure and flow rate, and provides real-time views of gradient composition and column backpressure. Plus, Eksigent's software engineers placed special emphasis on integrating control of the NanoLC-Ultra systems into the major mass spectrometry software packages. Drivers are currently available for Thermo Electron's Xcalibur® (see Figure 4), Bruker Daltonics' HyStar® and ABI/Sciex Analyst® software.

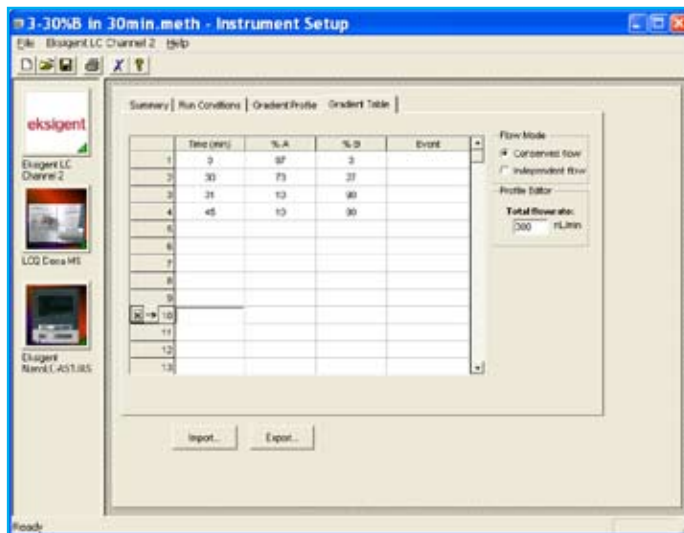
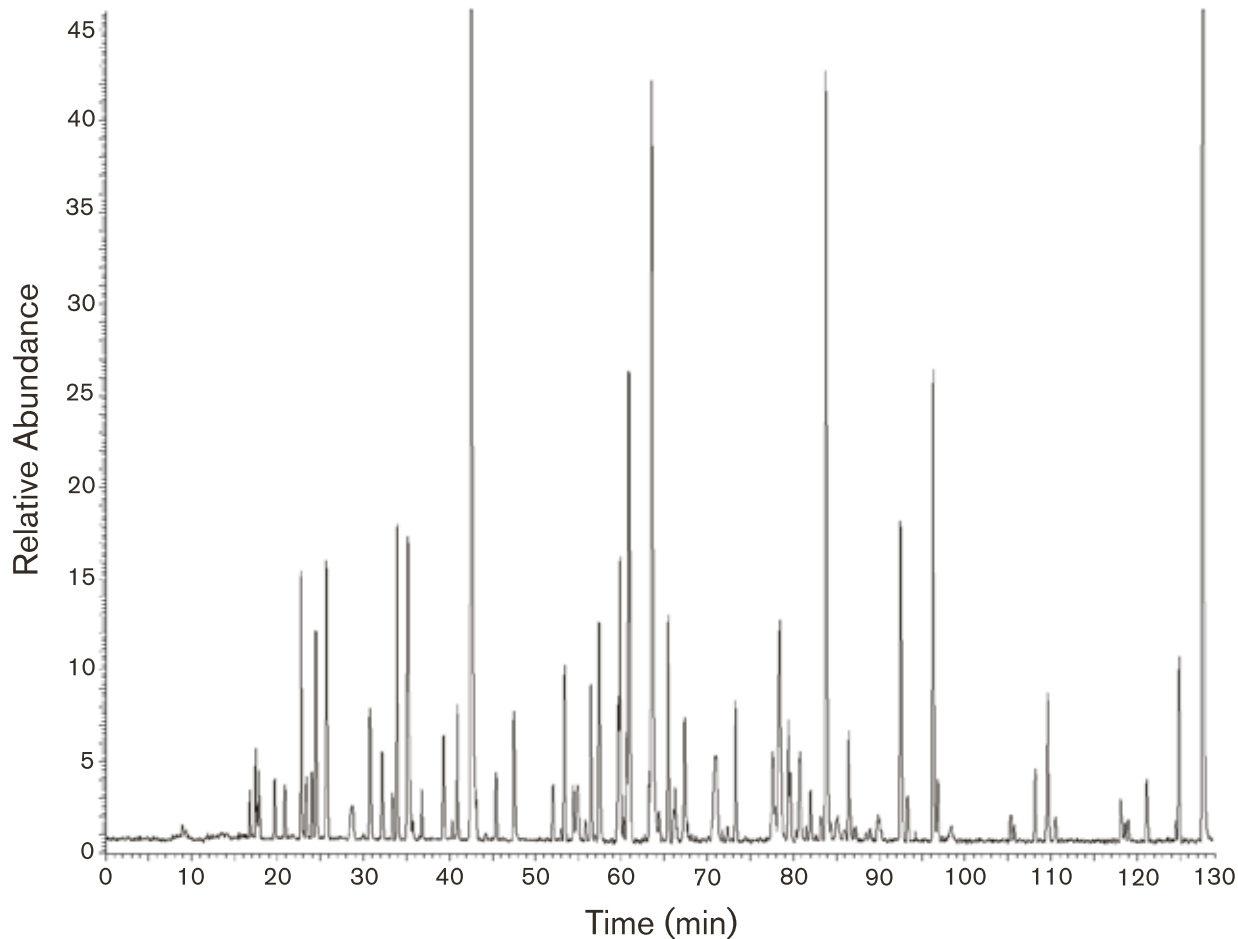


Figure 4. Software integration under Thermo Electron's Xcalibur.

Figure 3. High resolution separation of a BSA tryptic digest.

Separation of a BSA tryptic digest on a 75  $\mu$ m ID x 80 cm column packed with 3  $\mu$ m C18 stationary phase. Back pressure was 9,000 psi. The peak capacity for this separation is approximately 250.



## Flexible system configurations for proteomics

Currently, four configurations of the NanoLC-Ultra system are available for proteomics researchers:

- NanoLC-Ultra 1D,
- NanoLC-Ultra 1Dplus,
- NanoLC-Ultra 2D,
- and NanoLC-Ultra 2Dplus.

The NanoLC-Ultra 1D system contains one binary gradient pump, allowing for direct on-column injections. The NanoLC-Ultra 1Dplus system adds an isocratic loading pump for sample enrichment experiments in which sample is loaded onto a trap column prior to analysis on a NanoLC column.

NanoLC-Ultra 2D systems include all of the features of the 1D, plus the added capability of automated 2-dimensional chromatography. The NanoLC-Ultra 2D (two gradients) system and NanoLC-Ultra 2Dplus system (two gradients and one isocratic loading pump) are designed for advanced applications such as on-line 2D LC (see Figure 5) or high throughput set-ups using two nanoLC columns.

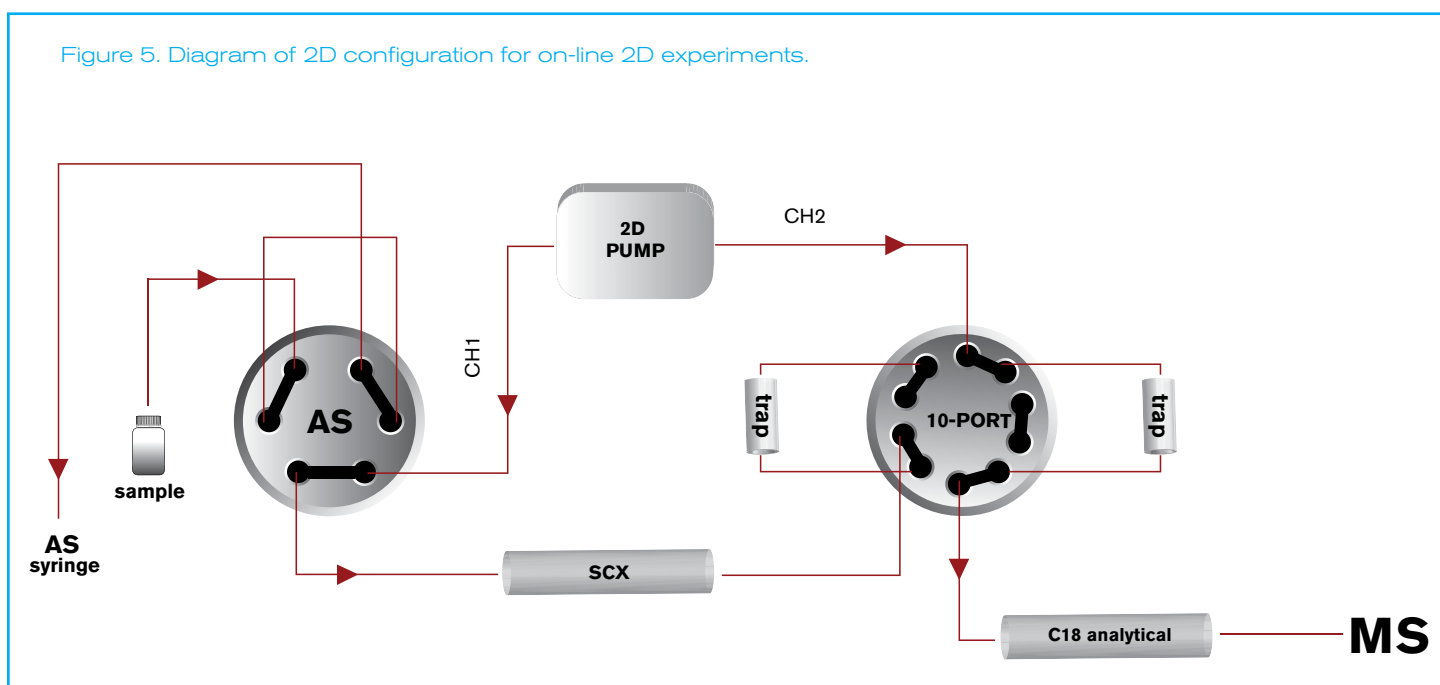
All NanoLC-Ultra 2D and 2Dplus systems are available with one or two built-in 10-port valves. NanoLC-Ultra 2D and 2Dplus system can be configured to run in either 2-dimensional separation mode or as two parallel, completely independent binary gradient HPLC systems.



### AS-2 autosampler

The new AS-2 Autosampler stacks together with the NanoLC-Ultra and both are operated by our Eksigent Control Software or through integration with the MS software. The flexible and easy-to-use autosampler employs a 2.4 ul volume needle for injection of small volumes without sample losses. The needle is coated with a proprietary inert coating on both the inside and outside to prevent sample losses due to adsorption. The sample compartment is completely enclosed to ensure temperature control down to 4°C. Capacity is two microtiter plates or 96 vials. Using two different wash positions, the needle is washed inside and outside to reduce carry-over. The 6-port injection valve has port-to-port volumes of <25 nl, and is rated to 10,000 psi. A proprietary coating on the valve stator and rotor assures excellent valve reliability and reduces sample adsorption.

Figure 5. Diagram of 2D configuration for on-line 2D experiments.



## Ordering information

p/n	system	10-port valves	loading pump	gradient 1	gradient 2
950-00055	NanoLC-Ultra 1D	N/A	N/A	50-500 nl/min	N/A
950-00056	NanoLC-Ultra 1Dplus	1	1-30 µl/min	50-500 nl/min	N/A
950-00057	NanoLC-Ultra 2D	1	N/A	1-10 µl/min	50-500 nl/min
950-00058	NanoLC-Ultra 2D	1	N/A	50-500 nl/min	50-500 nl/min
950-00059	NanoLC-Ultra 2D	2	N/A	1-10 µl/min	50-500 nl/min
950-00060	NanoLC-Ultra 2D	2	N/A	50-500 nl/min	50-500 nl/min
950-00061	NanoLC-Ultra 2Dplus	2	1-30 µl/min	50-500 nl/min	50-500 nl/min
620-00142	AS-2 autosampler	N/A	N/A	N/A	N/A

## System specifications

### NanoLC-Ultra

Dimensions	14" x 24" x 18" (W x D x H)
Weight	Up to 70 pounds (depending on configuration)
Working temperature	15° to 30°C
Power	100-240 V AC, 400 VA
Maximum pressure	10,000 psi
Standard flow rate ranges	
Loading pump	1-30 µl/min
Nano gradient	50-500 nl/min (up to 1000 nl/min at reduced maximum pressure)
Micro gradient	1-10 µl/min (up to 20 µl/min at reduced maximum pressure)
Flow rate accuracy	< 1%
Flow rate precision	< 0.5% @ 200 nl/min
Gradient accuracy	< 1% @ 200 nl/min
Gradient delay	< 25 nl
Column oven	Ambient + 5°C to 40°C (±1 °C)
Built-in 10-port valve	<ul style="list-style-type: none"> <li>▪ 1/32" connection; 0.1 mm bore</li> <li>▪ Port-to-port volume &lt; 25 nl</li> <li>▪ Max. pressure 10,000 psi</li> <li>▪ Stainless Steel with proprietary coating on valve stator</li> </ul>
Wetted parts	Stainless Steel, Titanium, PEEK, Fused Silica, FEP, proprietary inert coating
I/O	1 x USB for instrument control 2 x RS232 serial output Each pump channel <ul style="list-style-type: none"> <li>▪ 2 x TTL in</li> <li>▪ 4 x TTL out</li> </ul> AD converter
Instrument control	Eksigent software with plug-ins for Thermo Electron's Xcalibur®, Bruker Daltonics' HyStar® and ABI/Sciex Analyst® software

### AS-2 high pressure autosampler

Dimensions	14" x 24" x 14" (W x D x H)
Weight	46 pound
Working temperature	15° to 30°C
Power	100-240 VAC; 200 VA
Maximum pressure	10,000 psi
Capacity	Two microtiter plates (96 high/low or 384 low) or 48/12 sample vial racks
Injection valve	<ul style="list-style-type: none"> <li>▪ 1/32" connection; 0.1 mm bore</li> <li>▪ Port-to-port volume &lt; 25 nl</li> <li>▪ Max. pressure 10,000 psi</li> <li>▪ Stainless Steel with proprietary coating on valve stator and rotor</li> </ul>
Injection volume	Programmable from 100 nl to 10 µl with standard 10 µl loop. Larger injection volumes possible with optional loop.
Dispenser syringe	25 µl standard; optional : 100 µl, 250 µl
Injection volume reproducibility	<ul style="list-style-type: none"> <li>▪ Full loop: RSD &lt; 0.8%</li> <li>▪ Partial loop: RSD &lt; 1%</li> <li>▪ µl-pick-up: RSD &lt; 2%</li> </ul>
Carry-over	< 0.05%
Sample tray cooling	4°C to ambient -3°C (±2 °C)
Wetted parts	Stainless Steel, PTFE, PEEK, Glass, Teflon, TEFZEL, fused silica, proprietary inert coating
I/O	<ul style="list-style-type: none"> <li>▪ RS232 serial port for instrument control</li> <li>▪ 1 programmable relay output</li> <li>▪ 2 programmable TTL inputs</li> </ul>
Instrument control	Eksigent software with plug-ins for Thermo Electron's Xcalibur®, Bruker Daltonics' HyStar® and ABI/Sciex Analyst® software



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Eksigent is creating new possibilities for life science research, drug discovery & development, and medical devices with its innovative MicroFlow™ and NanoFlow™ fluid delivery systems.

Eksigent's LC systems deliver dramatic increases in analysis speed, throughput, and sensitivity. Eksigent's drug delivery systems bring new levels of precision to portable drug delivery.

Today, leading research, pharmaceutical, and biotechnology firms around the world use Eksigent's innovative solutions.