Thermo Scientific EASY-nLC 1000

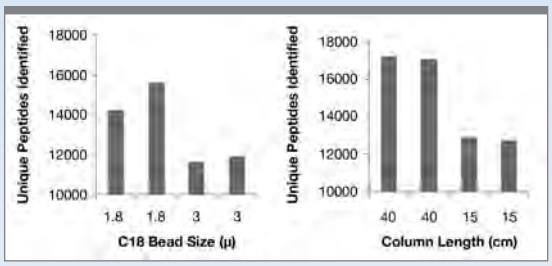
Effortless, Split-free Nano-flow UHPLC for Top Performance in LC-MS

Effortless Split-free, Nano-flow Performance at Ultra High Pressure

The workflow is extremely simple with seamless MS integration for instant trouble-free operation.

The Thermo Scientific EASY-nLC 1000 is a split-free, nano-flow liquid chromatograph optimized for separating biomolecules such as proteins and peptides at ultra high pressures up to 1000 bar or 15,000 psi. The instrument seamlessly integrates with various mass spectrometers, securing instant trouble-free operation. EASY-nLC 1000 is a fully integrated LC-system with a binary nano-flow gradient pump, a cooled autosampler, switching valves and high precision flow sensors for accurate solvent control before high-pressure mixing.

The market leading operating pressure of the EASY-nLC 1000™ system offers an unprecedented choice of columns and is designed to help researchers achieve top performance in LC-MS.



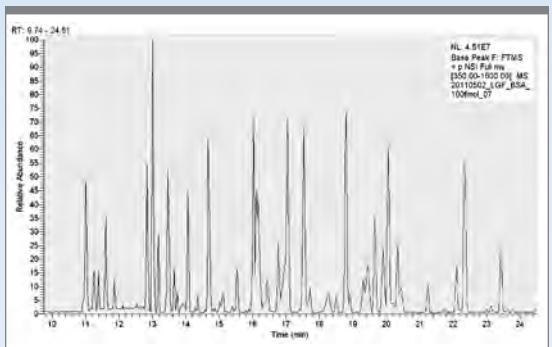
More Identifications

Many proteomic scientists focus on as many reliable peptide identifications as possible, often involving the use of longer columns or smaller beads that calls for higher pressure ratings.

Optimizing parameters may contribute as much as 30% more identifications, as illustrated in the histogram. Here experiments are compared; respectively 3 µm with 1.8 µm beads and 40 cm with 15 cm columns.

Improved number of identifications are equally important for research and discovery. In shotgun proteomics experiments on complex mixtures, those advances are also assisted by the improved performance from modern MS technologies such as the Thermo Scientific LTQ Orbitrap system.

The drive towards more identifications has previously concentrated on longer, more shallow gradients. With the introduction of the EASY-nLC 1000 system, these results can be obtained with shorter gradients on UHPLC columns with 50 µm inner diameter packed with 1.9 µm beads, that retain superior separation and deliver razor sharp peaks with FWHM in the range of 2-3 seconds.



Method:
- 45 cm length
- 100 fm BSA peptides

Sharper Peaks – Better Separation

Split-free, ultra high pressure nano-flow LC allows for almost infinite freedom when:

- Matching inner diameter to optimal linear velocity for each resin
- Selecting column length and smaller beads to improve separation
- Reducing column ID to increase analyte concentration for nano-electrospray ionization and improved detection sensitivity

Optimizing these factors contribute to improved chromatographic separation, and together they yield more peptides analyzed and more proteins identified. The EASY-nLC 1000 system allows you to optimize these factors within a wide range of nano-flow and pressure ratings.

An example of sensitivity optimization is shown in the BSA digest chromatogram. Here the beads are the same as used in classic nano-HPLC applications, the column is optimized in both length for better separation and internal diameter for higher sensitivity.

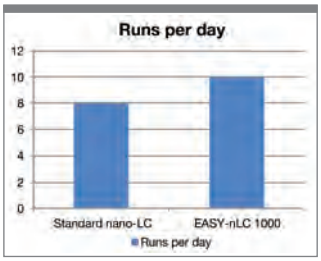
"Using the EASY-nLC 1000 system has increased our throughput by 25%"

Professor Matthias Mann, Max Planck Institute for Signal Transduction, Germany

EASY-nLC 1000 vs Standard nano-LC

Instrument Parameters

- **Max loading pressure 980 bar vs 280 bar**
- **Pre-gradient time < 25 min vs about 1 hr**
- **Same setup (140 min MS acquisition)**
- **10 runs per day vs 8 runs per day**

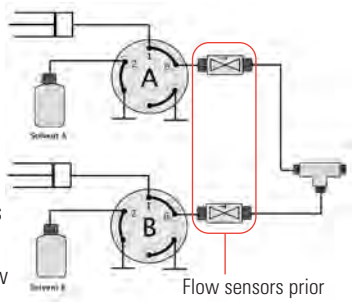


Excellent Reproducibility in Complex Samples

Highly reproducible chromatography is key to basic confidence in the generated data and also critical for experiments like:

- Quantification studies, both absolute and relative quantification
- Label-free experiments

Due to its accurate dual in-line nano-flow sensors, pulsation-free pumps and innovative software control, the EASY-nLC 1000 system is one of the most reliable and reproducible instruments today. Retention time variations are routinely below 0.4% RSD. This performance will also hold in very complex samples, as evidenced in the study shown below.



Flow sensors prior to UHPLC mixing enables precise real-time control

Proven Performance

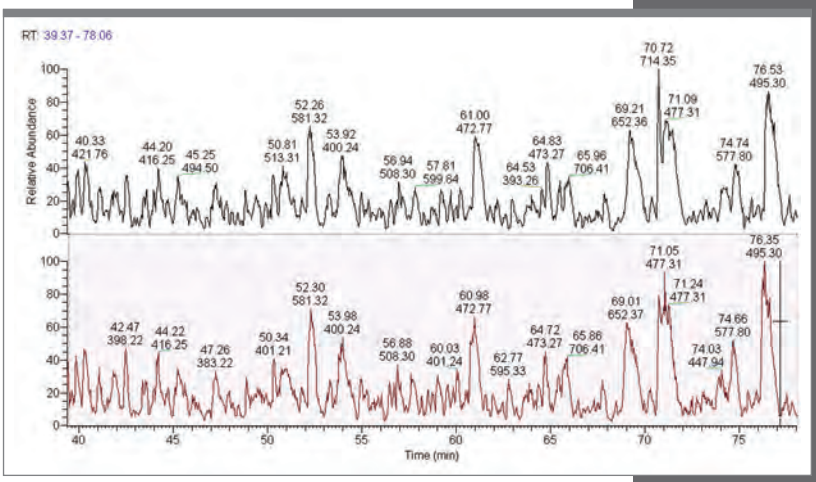
Multiple HeLa Cell Lysate Injections

HeLa cell lysates are very complex samples. In this experiment, 4 hour gradients were used to ensure good separation for efficient peptide analysis and subsequent protein identification.

The retention time deviation for some key peaks were observed to be less than 40 seconds, corresponding to an RT RSD of approximately 0.3%.

Instrument Parameters

- **Thermo Scientific Orbitrap Velos**
- **240 min 5%–40%**
- **Proxeon ES070 nano-ES source**



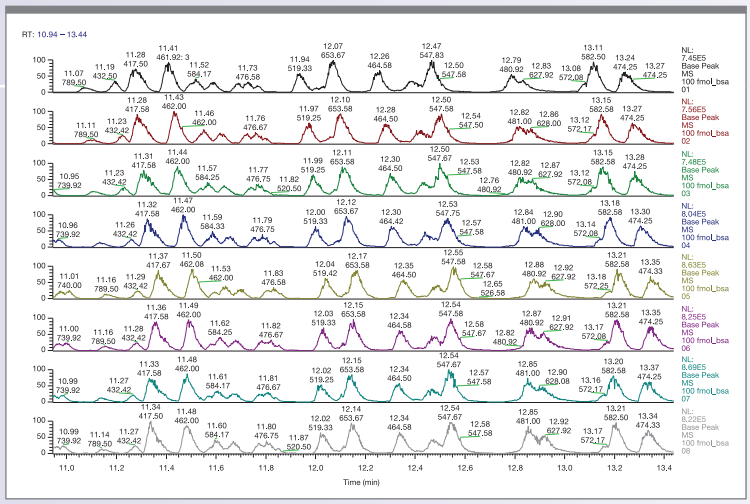
Multiple Tryptic Digest BSA Injections

Tryptic digest BSA is probably the most widely used proteomics standard, since it's readily available and has a distinct profile with number of very characteristic peaks that are easily recognized, providing an excellent basis for statistical analysis.

In this example short gradients were used to illustrate a number of different points besides impressive RT RSD (0.2%), namely sharp peaks (2.1s) and high column peak capacity.

Instrument Parameters

- **Thermo Scientific LTQ Velos**
- **1-column setup (10 cm, 50 µm ID, 1.9 µm C18)**
- **5–35% ACN over 10 min**
- **Thermo Scientific Nanospray Flex Ion Source**



Unrivaled Ease of Use and Productivity

The flow system is split-free for better reproducibility and simpler architecture.

Two flow sensors (one for each mobile phase) are placed immediately upstream of the high pressure mixing for accurate individual liquid measurements.

The pre-configured set-up of the EASY-nLC 1000 system minimizes the number of connections that has to be made by the operator. Only two unions have to be made to connect the column(s) to the eluent flow line and a waste/venting line. Power, contact closure and LAN are connected on the back. The factory configured flow paths minimize dead volumes and risk of internal connection leaks.

The touch screen display is the main interface. Method and batch setup is made in simple chromatography terms and using a wizard-like workflow.

Each unit is tested with a MS and required to pass a strict QC protocol prior to shipping. Units are shipped configured and ready for use. Installation takes hours rather than days and can be done by non-expert technicians since most tuning steps are performed by software scripts.

Long Lifetime Valves

Identical UHPLC valve stacks and polished stators with a proprietary coating ensure long trouble-free operation of the switching valves.

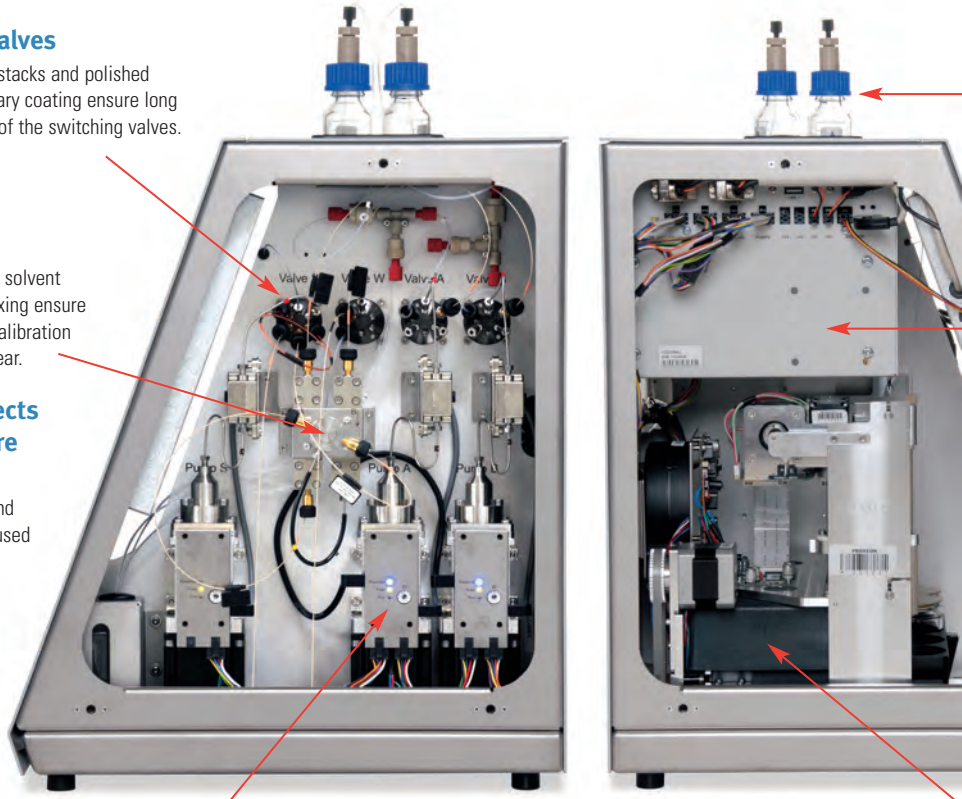
Accurate Gradient Delivery

Having dual in-line sensors measuring the solvent flow immediately before high-pressure mixing ensure accurate delivery of each mobile phase. Calibration points typically remain stable for over a year.

Intelligent Flow Control Protects Against System Over-pressure

IFC implements rapid build-up of pressure and flow to obtain optimal equilibration and sample loading. It avoids interruptions caused by exceeding maximum column pressure limits and allows analysis to continue despite increasing column back pressure.

- Both flow and pressure are monitored
- Ensures that the true volume is delivered
- Maximum pressure is user settable



Low Maintenance Pumps

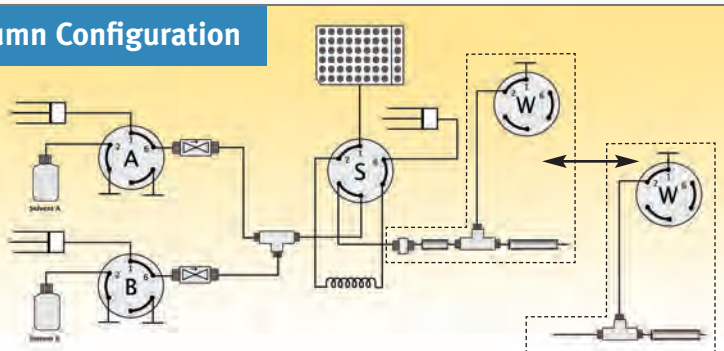
The pulsation-free direct drive pump design minimizes the need for seal replacements, just as wear and tear on valves and fittings are significantly reduced.

Easily Switch between 1 or 2 Column Configuration

Using two columns is great for robustness and loading speed meaning higher throughput.

Using only a single analytical column improves sensitivity at the cost of loading speed.

Changing between the two different configurations was never easier – simply connect or remove a trapping pre-column between the column out flow line and the venting tee.



Robust and Intelligent Maintenance

Robustness is excellent due to the minimal number of moving parts.

The direct drive syringe pumps are completely pulsation-free, thereby minimizing wear and tear on seals, valves and fittings. Tuning is automated and good robust chromatographic performance is obtained with preinstalled default methods. Intelligent and built-in scripts automate most maintenance requirements and can in most cases be scheduled for unattended execution.

Service is made easy by a number of design innovations. Side covers are removed in seconds and give fast and easy access to all components. The system design has been optimized with a minimum number of components and the modularity enables rapid parts exchange.

*

"Using the EASY-nLC 1000 system we were able to detect 30% more peptides using longer columns and smaller beads"

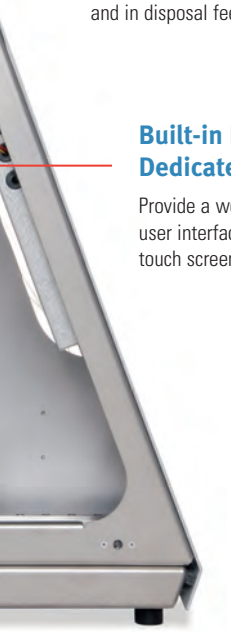
Prof. Jens Andersen, SDU CEBI, Odense, Denmark

Minimized Solvent Usage

The 2 × 25 mL solvent bottles is sufficient to run 24/7 for up to 3 weeks. This is a double saving, both in purchasing the high-grade LSMS solvents and in disposal fees afterwards.

Built-in PC and Dedicated Software

Provide a work-flow oriented user interface controlled by touch screen.

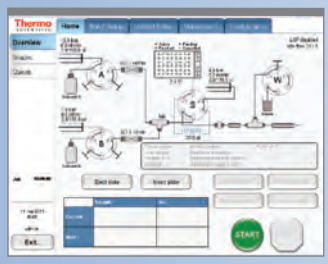


Enclosed, Refrigerated Sample Compartment

Well insulated design avoids condensation problems. Multiple sample adaptors enable wide selection of formats:

- 96 (8 × 12) or 384 (16 × 24) well plates
- 6 × 8 sample vials
- Adaptor for 6 × 8 and 3 × 8 microplate PCR strips

6 permanent vial positions are available for stock standard and blank solutions. The injection needle is flushed on the inside and washed on the outside between each sample for minimal carry-over. Additional solvent bottles for custom wash procedures support even more strict procedures.

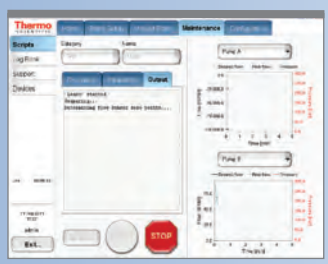


An **overview screen** provides complete system status at-a-glance. Information is available for:

- **Valve positions**
- **Pump solvent levels**
- **Flow sensor readings**
- **Autosampler temperature**
- **Sample queue processing**

Wizard-style, results-oriented **method development** only ask for the minimum number of relevant parameters. Detailed calculations are then handled automatically by the software. Inputs include:

- **Flow rates and maximum pump pressures**
- **Volumes**
- **%B mixing and step durations for the gradient**



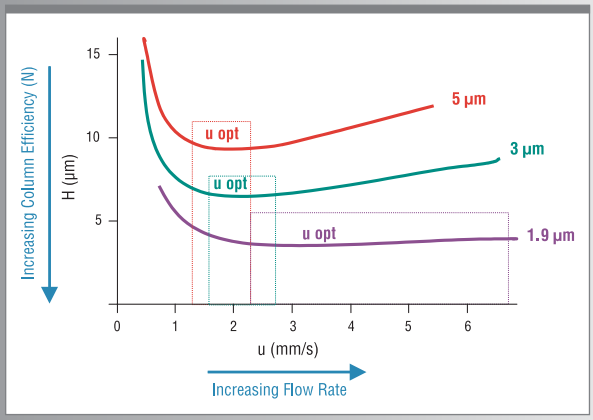
Intelligent and built-in scripts automate most maintenance and diagnostics tasks. Remote support over a network connection provides instant access to product specialists.

- **Automated leak test indicates actual position of leak**
- **System back pressure test quickly detects blockages**
- **Event logs help in troubleshooting and in monitoring system health and performance over time**

The same intuitive user interface is also used for quickly configuring the whole system. In addition to checking usage data, setting simple parameters, touch screen procedures are available for:

- **Backing up user methods and other critical data**
- **Calibration of flow sensors for different solvents**
- **Calibration of autosampler pick-up**
- **Checking contact closure operation**
- **Upgrading the system firmware**

Chromatographic Excellence at Nanoscale



Advantage of Small Particles

- **Using smaller chromatographic beads in the separation columns yields a number of benefits.**
  - ***Increased resolution from more theoretical plates***
  - ***Added binding capacity by having a greater surface area***
  - ***Wider flow range for optimal operation***
- **The fact that the required linear flow rate is often higher for smaller beads is typically satisfied by embedding the resin in capillaries with smaller inner diameters, often down to 50 µm or even 25 µm.**

Resin	ID	Flow	Length	Pressure	Theoretical Plates
1.9 µm	50 µm	310 nL/min	10 cm	650 bar	28800
3.0 µm	50 µm	200 nL/min	40 cm	670 bar	60400
3.0 µm	75 µm	300 nL/min	10 cm	150 bar	10000

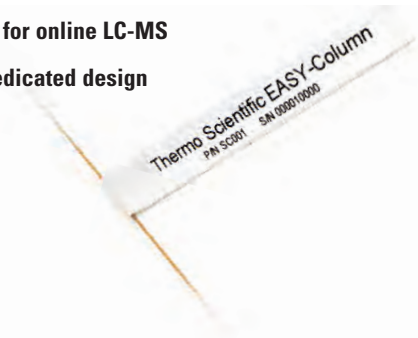
Unlimited Selections

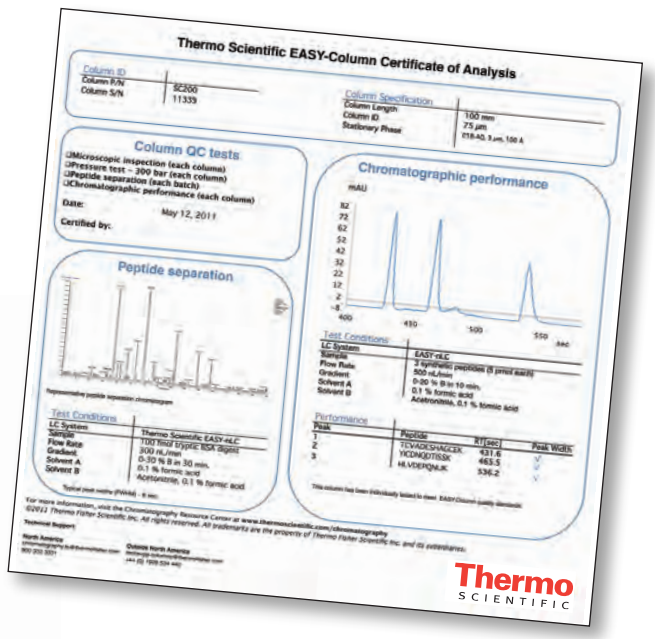
Different resins have different optimal linear flow rates, leading to a range of column geometries that utilise the full operating pressure range of the EASY-nLC 1000 system, with healthy safety margins.

Quality Control on Every Column

Using top quality media and components, and produced with a focus on design, simplicity and strict quality control, Thermo Scientific EASY-Column nano-bore capillary columns ensure top chromatographic performance.

- **Optimized for online LC-MS**
- **Simple, dedicated design**

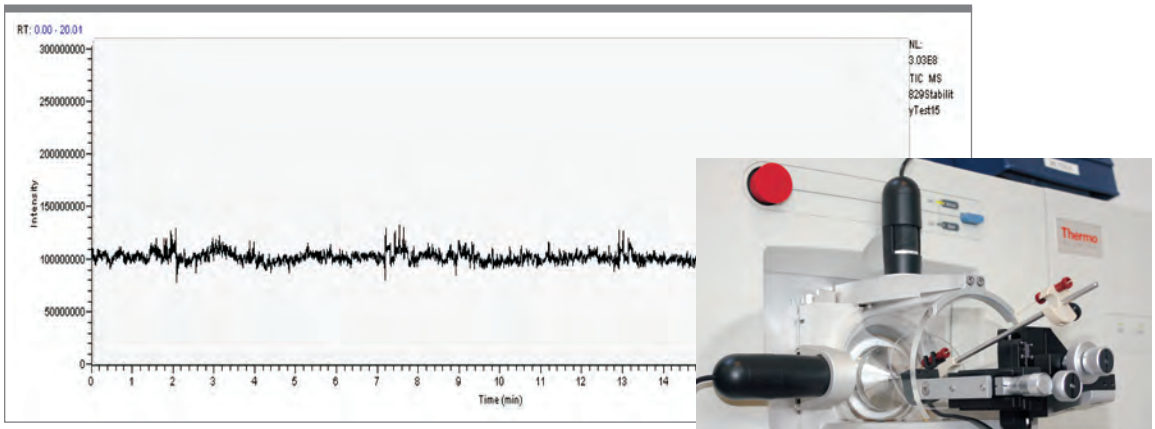




Ordering Information

	Description	Part Number
EASY-nLC 1000	Ultra high pressure, split-free, nano-flow LC	LC120
Nanospray Flex	Nano-electrospray ion source	ES071

Efficient Nano-electrospray Ionization for MS Analysis



Achieving the Highest Sensitivity

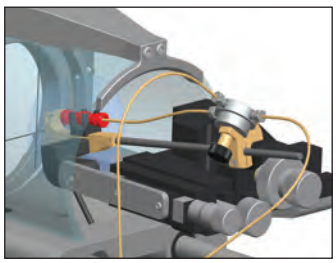
Mass spectrometry (MS) based discovery and characterization of biomolecules, such as proteins and peptides, has revolutionized biological research. Modern instrumentation provides high mass accuracy, resolution and sensitivity.

Thermo Fisher Scientific supplies researchers with reliable, high performance nano-electrospray ion sources for nano-flow electrospray ionization – the method of choice for MS analysis of proteins and peptides.

Developed with feedback from hundreds of valued customers, the Nanospray Flex™ Ion Source enables the use of nanoscale flow rates and maintains excellent spray stability to ensure efficient evaporation and ionization of liquid samples. Together with our leading MS systems, the Nanospray Flex Ion Source will help to achieve the highest possible sensitivity.

The Nanospray Flex Ion Source delivers a highly stable spray over a long run time, contributing significantly to the overall performance of a mass spectrometer. The figure clearly demonstrates the ability of the ion source to provide a continuously stable spray which prevents adverse effects on signal intensity and peak shape to ensure the highest sensitivity along with precise RSD values and calibration curves.

Mounting Columns

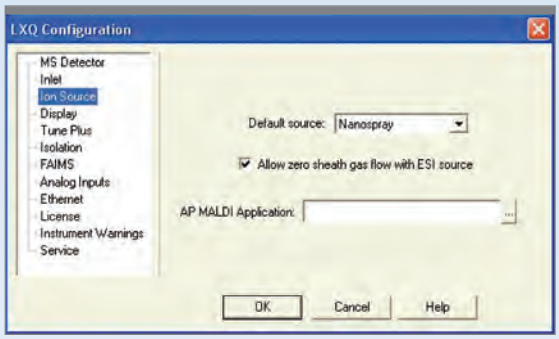


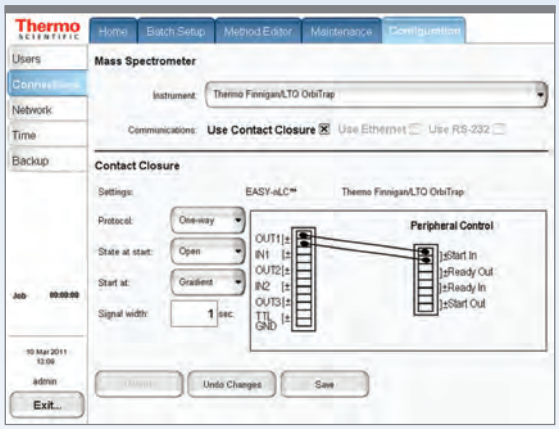
To ensure proper leak free connections for UHPLC columns, stainless steel tees and crosses are supplied with the EASY-nLC 1000 system.

To assist in handling these, a special bracket has been designed for the DirectJunction adapter on the Nanospray Flex source (ES071).

The bracket offers an efficient and elegant fixture for the column venting tee where the distance down to the spray emitter can be easily adjusted to accommodate different lengths of analytical columns.

The bracket is supplied together with the EASY-nLC 1000 system and attaches to the Nanospray Flex frame.





- **Effortless top performance in nano-flow LC/MS analysis with the EASY-nLC 1000 split-free HPLC instrument**
- **Both source and EASY-nLC 1000 are controlled from Thermo Scientific Xcalibur software**

Laboratory Solutions Backed by Worldwide Service and Support

Tap our expertise throughout the life of your instrument. Thermo Scientific Services extends its support throughout our worldwide network of highly trained and certified engineers who are experts in laboratory technologies and applications. Put our team of experts to work for you in a range of disciplines – from system installation, training and technical support, to complete asset management and regulatory compliance consulting. Improve your productivity and lower the cost of instrument ownership through our product support services. Maximize uptime while eliminating the uncontrollable cost of unplanned maintenance and repairs. When it's time to enhance your system, we also offer certified parts and a range of accessories and consumables suited to your application.

To learn more about our products and comprehensive service offerings, visit us at www.thermoscientific.com.

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