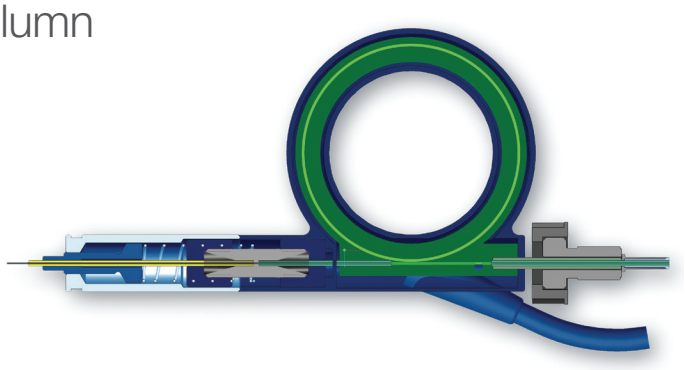
Raising the bar in proteomics

EASY-Spray C18, 75 cm x 75 µm Column



The Thermo Scientific™ EASY-Spray™ C18, 75 cm × 75 µm column offers greater peak capacity and hence increases the number of peptides in bottom-up LC-MS/MS analysis. Better peptide separation results in high peptide identification rates and identification of low abundant peptides.

Product highlights

- Higher peak capacity for enhanced separation of complex mixtures
- Use of longer gradients to maximize sequence coverage
- High resolution for protein identification
- Designed for TFA-free LC-MS analysis, minimizing ion suppression effects
- High pressure capability for fast loading and equilibration

Improved sensitivity in LC-MS analysis

The EASY-Spray C18, 75 cm column combines the ease-of-use of EASY-Spray with the advantages of the higher resolving power of longer columns containing the Thermo Scientific™ Acclaim™ PepMap™ RSLC C18 stationary phase. Through higher peak capacity, the number of identified peptides in a complex mixture can be increased without increasing analysis time. Moreover, the gradient length can be further extended to separate peptides and further improve identification, which is important in the LC-MS analysis of lower abundant proteins.

Product benefits

The EASY-Spray design is an easy-to-use “plug and spray” approach designed to improve usability and is an alternative to classic nano LC columns and sources. Integrating this technology with a 75 cm column further enhances performance by the ability to improve peak capacity using existing gradients, or by the use of longer gradients to further improve the separation of complex mixtures.

The EASY-Spray C18, 75 cm column can be operated at pressures of up to 1200 bar. Running at this pressure brings advantages in that column equilibration and sample loading can be done at higher flow rates, leading to improved productivity and reduced waiting time per injection.

Technology

The EASY-Spray C18, 75 cm column is packed with Acclaim PepMap C18 2 µm 100 Å particles and has a 75 µm inner diameter. It is used for the separation of digested peptides for the identification of proteins in proteomics applications. Acclaim PepMap is designed for TFA-free analysis, which minimizes ion suppression effects in mass spectrometry experiments.

Applications

Improved peak capacity

The separation of complex peptide mixtures and their subsequent identification is crucial in the field of proteomics. The quality of the separation and the ability to maximize peak resolution, over a large range of peptide fragment sizes, is dependent on the separation efficiency of the columns and the choice of gradient elution profiles.

Higher peak efficiency produces greater sensitivity, allowing detection of more peaks with greater confidence. The overall effect is that separations will give a greater sequence coverage from each analysis that requires the use of high resolution LC-MS/MS, allowing collection of more data which leads to more rapid and precise protein sequence identification.

Figure 1 highlights that the 75 cm column (P/N ES905) gives significantly reduced Peak Width at Half Height (PWHH), compared to the 50 cm column (P/N ES903) for a mixture of Peptide Retention Time Calibration (PRTC) compounds. This allows better quantitation with lower limits of detection.

Reproducibility and coverage

Multiple injections of the HeLa/ PRTC solution (n=5) were compared for the 50 cm and 75 cm columns. The data was processed and the average numbers of peptides identified were compared to highlight any differences in sequence coverage as shown in Table 1.

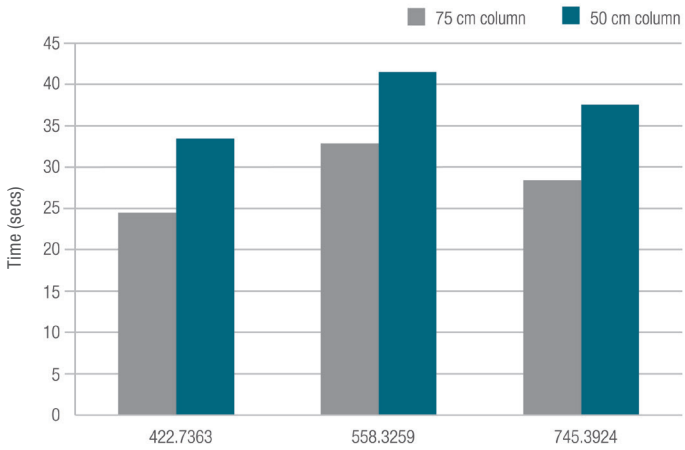


Figure 1. Reduced PWHH with PRTC peptides *m/z*.

The higher resolving power of the 75 cm column is indicated in Figures 2 and 3. This shows the TIC observed after the analysis of HeLa Digest using a 50 cm and a 75 cm column, while using the same gradient profile for both columns.

A significant increase in the number of identified peptide groups of over 25% on the 75 cm column was observed. This allows the longer 75 cm column to identify up to 5200 more peptides in a single run, compared to the 50 cm version.

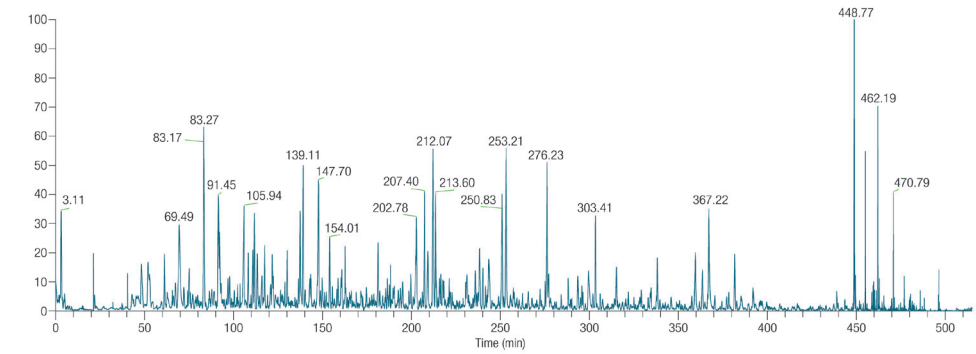


Figure 2. HeLa Digest TIC on a 50 cm column (1 µL injected).

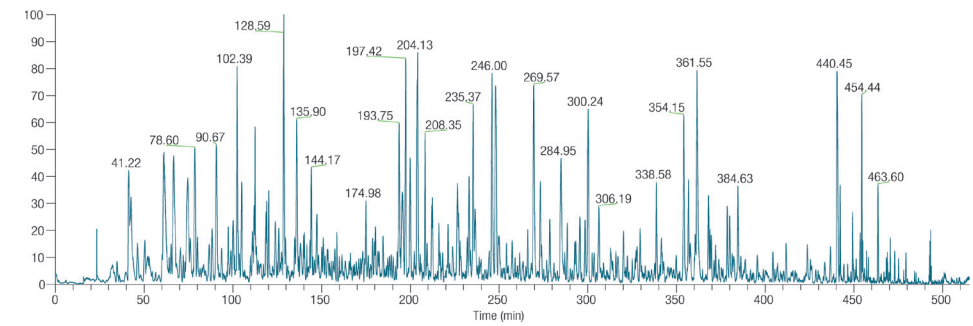


Figure 3. HeLa Digest TIC on a 75 cm column (1 µL injected).

thermo scientific

Table 1. Peptide and protein coverage search.

Column	PSM	Peptide groups	Protein groups
75 cm (P/N ES905) Average	170574	40230	5070
50 cm (P/N ES903) Average	118050	32013	4828
Improvement % of 75 cm column vs 50 cm column	43.40%	25.67%	5.01%

Operational specifications

Parameter	Recommendation
Typical applications	Peptide analyses
Base material	Spherical silica, 2 µm 100 Å pores
Chemistry	C18, endcapped
Column dimensions	75 µm i.d. × 75 cm
Recommended flow	200–400 nL/min
Recommended temperature	25–60 °C
Maximum pressure	1200 bar
Recommended sample quantity[1]	2 µg
Maximum sample capacity[2]	10 pmol
pH stability	2.0-8.0
Solvent compatibility	All common reversed phase solvents

[1] The recommended sample quantity of the Acclaim PepMap 75 µm i.d. columns is given in weight amount of a protein digest of bovine serum albumin.
[2] The maximum sample capacity is measured for tryptic peptides from cytochrome c digest tolerating a maximum 10% increase in peak width.

Ordering information

Product	Particle size	Part number
EASY-Spray C18, 75 cm x 75 µm	2 µm	ES905

Find out more at **thermofisher.com/EASYspray**

ThermoFisher SCIENTIFIC