

Ascentis® Express Peptide ES-C18 Column Care & Use Sheet

Ascentis Express Peptide ES-C18 Description

Ascentis Express Peptide ES-C18 is a high-speed, high-performance liquid chromatography column based on a new 160 Å Fused-Core™ particle design. The Fused-Core particle provides a thin porous shell of high-purity silica surrounding a solid silica core. This particle design exhibits very high column efficiency for high MW solutes (up to 20 kDa) due to the shallow diffusion paths in the 0.5-micron thick porous shell and the small overall particle size of 2.7-microns. The non-end-capped, sterically protected C18 bonded phase of Ascentis Express Peptide ES-C18 provides a stable, reversed phase packing with a pore structure and pore size that is optimized for reversed-phase HPLC separations of peptides and polypeptides, using typical acidic mobile phases favored for protein structure-function and proteomic applications.

Column Characteristics

The Fused Core particle has a surface area of ~80 m²/g and an average pore size of 160 Å. The Fused Core particles are 30% to 50% heavier than commercially available totally porous particles due to the density of the solid cores. Therefore, the effective surface area per column is similar to columns packed with totally porous particles having surface areas of 150 - 200 m²/g.

Operation Guidelines

- The direction of flow is marked on the column label.
- Reversed flow may be used to attempt removal of inlet pluggage or contamination.
- A new column contains a mixture of acetonitrile and water. Initial care should be taken to avoid mobile phases that are immiscible with this mixture or could cause a precipitate.
- Ascentis Express Peptide ES-C18 columns have been application tested using low pH mobile phases at operational temperatures up to 100 °C, and have been qualified to exhibit very long column life at 60 °C.
- Mobile phase pH for Ascentis Express Peptide ES-C18 columns is best maintained in the range of pH = 1 to 8 for maximum column stability.
- Ascentis Express Peptide ES-C18 columns are stable to operating pressures up to 600 bar (9000 psi).
- Separations of complex peptide samples, such as tryptic digests of proteins, are typically accomplished using the Ascentis Express Peptide ES-C18 columns with gradient elution from 2% acetonitrile to 50% acetonitrile in the mobile phase. Although the column is compatible with 100% aqueous mobile phases, it is always advisable to check retention reproducibility of early eluting sample components, when using very low organic content (<2%) at the start of a gradient.

Column Care

To maximize column life, ensure that samples and mobile phases are particle-free. The use of guard columns or an in-line filter with 0.5-micron porosity between the sample injector and the column is highly recommended. The 2-micron porosity frits on Ascentis Express Peptide ES-C18 columns are less subject to pluggage than are the 0.5-micron frits typically used with other small-particle columns. Should the operating pressure of the column suddenly increase beyond normal levels, reversing the flow direction of the column may be attempted to remove debris on the inlet frit.

To remove strongly retained materials from the column, flush the column in the reverse direction with very strong solvents such as 100% of the organic component of the mobile phase in use. A mixture (95/5 v/v) of dichloromethane and methanol is often effective at removing lipidic contaminants and certain detergents. Extreme cases may require the use of very strong solvents such as dimethylformamide (DMF) or dimethylsulfoxide (DMSO).

Column Storage

Long-term storage of silica-based, reversed-phase columns is best in 100% acetonitrile. Columns may be safely stored for short periods (up to 3 or 4 days) in most common mobile phases. However, when using buffers, it is best to remove the salts to protect both the column and the HPLC equipment by flushing the column with the same mobile phase without the buffer. Before storing the column, the end-fittings should be tightly sealed with the end-plugs that came with the column.

Applications

The Ascentis Express Peptide ES-C18 columns are best utilized with mobile phases that are mixtures of acetonitrile and water or methanol and water. Higher levels of the organic solvent component will typically reduce the retention of the sample compounds. Using elevated temperatures (e.g., 40 - 100 °C) will reduce the viscosity of the mobile phase and allow the use of faster flow rates and lower column pressure for high sample throughput. Gradient-elution techniques using 5-10% organic component as the initial mobile phase and increasing to 100% organic component as the final mobile phase often can affect separations of complex sample mixtures in minimal time.

Ionizable compounds, such as acids and bases, are generally best separated with mobile phases buffered at pH of 2 to 3. The use of 10-50 mM buffers is always recommended for optimum results and long-term stability when separating ionizable compounds.

Ascentis Express Peptide ES-C18 columns utilize a steric-protected C18 bonded-phase with extremely high resistance to acid-catalyzed hydrolysis of the siloxane bond that attaches the C18 chain to the surface. Thus, the combination of low pH and elevated temperature operation of the column is well tolerated. Peptide separations are efficiently conducted using low pH mobile phase modifiers, often at 0.01-0.1% concentration, most popularly employing trifluoroacetic acid (TFA), and the related perfluorocarboxylic acids, pentafluoropropionic acid (PFPA) and heptafluorobutyric acid (HFBA). These acids exhibit desirable low UV transparency, volatility, and peptide ion pairing properties. Additional opportunities for low pH operation include the normal short chain carboxylic acids, formic acid and acetic acid, as well as mineral acids, such as phosphoric acid (0.001-0.02 M).

Guidelines for Low-Volume Columns

High performance columns with small internal volumes (shorter lengths, internal diameters < 3 mm) are being increasingly used for high-speed separations, especially with mass spectrometers. These low-volume columns generate peaks having considerably less volume than those eluting from columns of larger dimensions (e.g., 15 cm x 4.6 mm I.D.). The efficiency of separations performed in low-volume columns is highly dependent on the HPLC system having components designed to minimize band spreading. All low-volume columns perform best when used with proper attention to the following factors:

- **Detector** – Flow cell volumes should be < 2µL to properly sense and integrate the very fast peaks that can elute from low-volume columns, the detector response time should be set to the fastest level (~ 0.1 second) to allow integration of signal by software of at least 20 points across the narrowest peak.
- **Injector** – The injection system should be of a low-volume design (e.g., Rheodyne® Model 8125). Auto-samplers will often cause band-spreading, but may be used for convenience with the expectation of some loss in column efficiency.
- **Connecting Tubing** – The shortest possible lengths of connecting tubing with small internal diameters (≤ 0.005-inch, 0.12 mm I.D.) must be used to connect the column to the injector and the detector cell.
- **Peak Retention** – As retention is increased, the peak volume increases, decreasing extra-column band spreading caused by components of the instrument.
- **Sample Injection** – For isocratic separations, the volume of sample injected should be kept as small as possible (≤ 2 µL) in a solvent weaker than the mobile phase. Sample volumes are less critical for gradient separations, and a larger volume is possible if the sample is dissolved in a weak solvent.

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