BIOshell™ A160 Peptide CN, 2.7 µm Column Care & Use Sheet

Description

BIOshell™ A160 Peptide CN is a high speed, high performance liquid chromatography column based on Fused-Core® particles with 160 Å pores. The Fused-Core particle provides a thin porous shell of high purity silica surrounding a solid silica core. This particle design exhibits excellent 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 micron. The highly endcapped, sterically protected di-isopropyl-cyanopropylsilane bonded phase of BIOshell A160 Peptide CN columns provide a stable, semi-polar reversed phase packing with a pore structure and pore size that is optimized for reversed phase HPLC separations of peptides and small proteins. BIOshell A160 Peptide CN columns provide an alternative selectivity to BIOshell A160 Peptide C18 columns for protein structure/function and proteomic applications.

Column Characteristics

A printed report including the actual QC test chromatogram and a Batch Quality Assurance Test are enclosed with every column.

The Fused-Core particle has a surface area of ~ 80 m^2/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. The effective surface area per column is similar to columns packed with totally porous particles having surface areas of 150 - 200 m^2/g.

Operation Guidelines

- The preferred direction of flow is marked on the column label.
- Reversed flow may be used to attempt removal of inlet blockage or contamination.
- A new column contains 100% acetonitrile. Initial care should be taken to avoid mobile phases that are immiscible with this solvent or could cause a precipitate.
- BIOshell A160 Peptide CN columns have been application tested using low pH mobile phases (pH 1 - 2.5) at operational temperatures up to 100 °C, and have been qualified to exhibit very long column life at 60 °C at intermediate pH (up to pH 6). The maximum recommended temperature for operation at pH 6 - 8 is 40 °C for long column life.
- BIOshell A160 Peptide CN columns are stable to operating pressures up to 600 bar (9000 psi).
- Separations of complex peptide samples on BIOshell A160 Peptide CN columns, such as tryptic digests of proteins, are typically accomplished with gradient elution from 2% 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 poorly retained (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 free of particles. The use of guard columns or an inline filter with 0.5 micron porosity between the sample injector and the column is highly recommended. The 2 micron porosity frits on BIOshell A160 Peptide CN columns are less subject to blockage than 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 from the inlet frit.

To remove strongly retained materials from the column, flush the column in the reverse flow 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

Use 100% acetonitrile for long term storage of silica based reversed phase columns. Columns may be safely stored for 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 endfittings should be tightly sealed with the endplugs that came with the column.

Safety

- **HPLC columns are for laboratory use only. Not for drug, household, or other use.**
- Users of HPLC columns should be aware of the toxicity or flammability of the mobile phases chosen for use with the columns. Precautions should be taken to avoid contact and leaks.
- HPLC columns should be used in well-ventilated environments to minimize concentration of solvent fumes.

Applications

BIOshell A160 Peptide CN columns are commonly operated by running a gradient from low (5%) to high percent (95%) acetonitrile at low pH. The use of elevated temperature improves sample throughput. The sterically protected CN bonded phase in BIOshell A160 Peptide CN columns allows long term operation of the column at low pH and elevated temperature. 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 mineral acids, such as phosphoric acid (0.001-0.02 M). Additional information on solvent selection and separation techniques can be found in Chapters Six, Seven, Eight, and Eleven in Practical HPLC Method Development, Second Edition, L.R. Snyder, J.L. Glajch, and J.J. Kirkland, (John Wiley & Sons, 1997).

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). 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 cells should be of low volume design (preferably <2 µL). To properly sense and integrate the often very fast peaks that elute from low volume columns, the detector response time should be set to the fastest level (~ 0.1 second) and the integration software should sample the detector signal at least 20 points per second.
- **Injector:** The injection system should be of a low volume design (e.g., Rheodyne® Model 8125). Autosamplers 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 ID) 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 the relative importance of extra column band spreading caused by components of the instrument.
- **Sample Solvent:** 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.

Ordering Information

For ordering information or for technical support on this product, visit the Sigma-Aldrich website at **sigmaaldrich.com**, contact the Sigma-Aldrich office or a designated distributor in your country.