Bio-Monolith Protein A Column

Data Sheet

About Bio-Monolith Protein A Columns

Bio-Monolith Protein A Columns are high-performance monolithic columns that offer all the advantages of a specially designed, continuous short polymeric bed. Their inherent features enable highly reproducible separation and quantitation of immunoglobulin G (IgG) from cell culture supernatants or human plasma, at extremely high speeds. These columns are prepacked in dedicated stainless steel housings and allow user-friendly (and quick) connections to HPLC equipment.

The information in this data sheet is being provided to ensure proper product care and maximal product lifetime.

Basic Characteristics

Catalog number	5069-3639
Column chemistry	Immunoaffinity; Protein A from *Staphylococcus aureus*
Matrix	Poly(glycidyl methacrylate -*co*- ethylene dimethacrylate) highly porous monolith
Matrix dimensions	Diameter: 5.2 mm; length: 4.95 mm; bed volume (CV): 0.10 mL
Dynamic binding capacity	> 8 mg hIgG/mL wet support (Conditions: hIgG 0.5 mg/mL, PBS buffer, pH 7.4, flow rate 1 mL/min)
Maximum loading capacity	0.4 to 0.5 mg
Working flow rates	Recommended: 0.2 to 1 mL/min (1 to 5 cm/min; 2 to 10 CV/min) Maximum: 2 mL/min (10 cm/min; 20 CV/min)
Maximum allowed pressure over the column	75 bar (7.5 MPa, 1100 psi) **WARNING: Do not exceed the maximum allowed pressure as this might seriously damage your column!**
Temperature stability	Working: 4 to 40 °C (39 to 122 °F) Storage: 4 to 30 °C (39 to 73 °F) **WARNING: Avoid prolonged use at elevated temperatures!**
Recommended pH	Working range 2 to 11 Cleaning in place 2 to 13

Taking Care of the Column

Regeneration

In order to regenerate the column, wash it with at least 2 mL (20 CV) of a 0.1 M buffer containing 1 M NaCl, pH 7.0 to 8.0 at 0.5 to 1.0 mL/min. After that, wash the column with at least 2 mL (20 CV) of a low-pH solution (for example, 10 mM HCl or 0.1 M glycine-HCl) at 0.2 to 0.5 mL/min. Re-equilibrate the column with 5 to 6 mL (50 to 60 CV) of the working mobile phase at 0.5 to 1.0 mL/min. For best results, this should be done at the end of each chromatographic run.

Cleaning in Place (CIP)

In some cases, the simple regeneration of the monolithic column is not enough. Sample molecules may not fully elute from the column or may even precipitate on the column. This build up of contaminants on the monolithic column may cause loss of resolution and binding capacity, increased back pressure, or a complete blockage of the column. A specific CIP protocol should be designed according to the type of contaminants that are present in your sample. Regular cleaning (when using pure samples) can be performed by following this procedure:

1. Wash the column with 1 to 2 mL (10 to 20 CV) of 0.1 M NaOH.

Note: Reverse the flow direction and use low-enough flow rates (0.2 to 0.5 mL/min) to expose the column to NaOH for several minutes.

2. Wash the column with 1 to 2 mL (10 to 20 CV) of deionized (DI) water at the working flow rate.
3. Wash the column with 1 to 2 mL (10 to 20 CV) of a concentrated buffer (for example, 0.1 M to 0.5 M phosphate buffer) in order to quickly restore the appropriate pH.
4. Re-equilibrate with at least 5 mL (50 CV) of the binding mobile phase (buffer) at the working flow rate.

If the impurities are highly hydrophobic or lipidic and are not easily removed from the column, you may also use other cleaning solutions like 2-propanol (up to 30%) or guanidine hydrochloride (up to 6 M). After using these alternative cleaning solutions, follow the steps previously described (see steps 1 through 4).

WARNING: When you wash the column with these cleaning solutions, always decrease the flow rate on the column in order to avoid generation of high pressures on the column that might exceed the maximum allowed pressure over the column.

Long-Term Storage

If the column will not be in use for more than two days, it should be washed with at least 1 mL (10 CV) of DI water and afterwards flushed with at least 2 mL (20 CV) of a 20% ethanol solution at the flow rate of 0.2 to 0.5 mL/min, sealed with column end-stoppers and stored appropriately (4 to 8 °C [39 to 46 °F]).

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Published in the USA
September 16, 2008
5973-1722

Agilent Technologies