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ProductInformation

Anhydrotrypsin - Agarose

Product Number **A 2206**
Storage Temperature 2-8 °C

Product Description
Agarose resin physical properties:
Molecular Weight Range Exclusion Limits -
Globular proteins: 6×10^4- 2×10^7
Dextrans: 3×10^4- 5×10^6
DNA: 872 base pairs
Wet bead diameter: 45-165 µm
pH Range: 4 - 10
Maximum pressure: 80 cm of H_2O
Maximum linear flow rate: 11.5 ml/cm^{2}h

Anhydrotrypsin-agarose is an affinity chromatography resin that selectively binds peptides with Arg, Lys, or S-aminoethylcysteine (AECys) at the C-terminus under mildly acidic conditions. It does not bind free amino acids or peptides which have Arg, Lys, or AECys at sites other than the C-termini.[1-4] Bound proteins can be eluted with 5 mM HCl, pH 2.5, or 100 mM acetic acid, pH 2.5.

Agarose-based resins melt upon heating to 40 °C, cannot be autoclaved, and the bead structure may be damaged upon freezing. This agarose resin is stable in aqueous solutions at pH 4 - 10. Use of dissociation media such as guanidine hydrochloride and urea, chaotropic salts such as KSCN, and oxidizing agents is not advisable because these reagents may disrupt the hydrogen bonds which stabilize the matrix. Sterilization can be done chemically.

Before packing a column, dilute the required amount of resin with equilibration buffer to form a thick slurry, about 75% of which is settled resin, then degas the slurry. The maximum operating pressures (shown above) should never be exceeded or bed compression will occur and the column can stop flowing. Pass 2-3 column volumes (CV) of equilibration buffer through the gel to equilibrate the bed. Elution may be achieved by gravity feed or through the use of a peristaltic pump.

More consistent flow rates and more reproducible separations are obtained with a pump. Agarose resins can be cleaned in the column or batchwise. Wash with at least 1 CV of 0.5 N NaCl in 0.1 N NaOH, then follow with 10 CV of water (or until the eluent is at neutral pH).

Precautions and Disclaimer
For Laboratory Use Only. Not for drug, household or other uses.

Storage/Stability
This resin is a suspension in 50 mM sodium acetate, pH 5.0, containing 20 mM $CaCl_2$ and 0.02% sodium azide. The suspension is stable at 4 °C for at least one year. Do not store the resin in a buffer such as HCl or formic acid solutions, pH 2.5 for extended periods.

References
1. Ishii, S., et al., Methods Enzymol., **91**, 378-383 (1983).
2. Kumazaki, T., et al., A novel method for selective isolation of C-terminal peptides from tryptic digests of proteins by immobilized anhydrotrypsin: application to structural analyses of the tail sheath and tube proteins from bacteriophage T4. Proteins, **1(1)**, 100-107 (1986).
3. Yokosawa, H., and Ishii, S., Immobilized anhydrotrypsin as a biospecific affinity adsorbent for the peptides produced by trypsin-like proteases. Anal. Biochem., **98(1)**, 198-203 (1979).
4. Kumazaki, T., et al., Affinity chromatography on immobilized anhydrotrypsin: general utility for selective isolation of C-terminal peptides from protease digests of proteins. J. Biochem. (Tokyo), **102(6)**, 1539-1546 (1987).

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