GSTrap 4B Columns

Product Information

Cat#No#	GST-097P

Product Overview

GSTrap 4B columns are prepacked Glutathione Sepharose 4B columns for convenient, high capacity one-step purification of glutathione S-transferase (GST) tagged proteins.

Description

GST-tagged proteins can be purified directly from pretreated bacterial lysates using GSTrap 4B. GST-tagged proteins are eluted under mild, nondenaturing conditions using reduced glutathione. The purification process preserves protein antigenicity and function. If desired, cleavage of the protein from GST can be achieved using a site-specific protease whose recognition sequence is located immediately upstream from the multiple cloning site on the pGEX plasmids. GST-tagged proteins can be detected using colorimetric or immunological methods. The resin, Glutathione Sepharose 4B, is also available as lab packages and is a good choice for scale-up. The columns can be operated with a syringe, peristaltic pump, or liquid chromatography system.

Characteristic

High binding capacity.
Mild elution conditions preserving protein antigenicity and function.
Easy one-step purifications of Glutathione S-Transferase (GST) tagged proteins resulting in high purity.
GSTrap 4B are designed to be used with a syringe, pump, or chromatography system, such as ÄKTA design.

Applications

GSTrap 4B columns are used for the purification of GST-tagged proteins from bacterial lysates and other glutathione S-tranferases or glutathione-dependent proteins. Mild elution conditions preserve protein antigenicity and function.

Maximum operating pressure

5 bar [0.5 MPa] (70 psi)

Sample preparation

The sample should be centrifuged and/or filtered through a 0.45 µm filter immediately before it is applied to

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the column. If the sample is too viscous, dilute it with binding buffer to prevent clogging the column. Less protein may bind to the medium due to a lower protein concentration in the sample.

Matrix
4% agarose
Average particle size
90 µm
Ligand
glutathione and 10-carbon linker arm
Ligand density
7 to 15 µmol glutathione/ml medium
Dynamic binding capacity
Approx. 25 mg recombinant glutathione Stransferase (Mr 26 000)/ml medium (protein dependent).
Recommended flow rate
< 4 ml/min
Recommended column height
25 mm
Chemical stability
All commonly used aqueous buffers, e.g., 1 M acetate pH 4.0 and 6 M guanidine hydrochloride for 1 h at room temperature.
CIP stability
4 to 13
Storage
4 to 30°C, 20% Ethanol
Binding buffer

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PBS, pH 7.4 (140 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, pH 7.4).
Elution buffer
50 mM Tris-HCl, 10 to 20 mM reduced glutathione, pH 8.0.
Cleaning-in-place
Removal of precipitated or denatured substances: Wash with 2 column volumes of 6 M guanidine hydrochloride, immediately followed by 5 column volumes of PBS. Removal of hydrophobically bound substances: Wash with 3 to 4 column volumes of 70% ethanol or 2 column volumes of 1% Triton X-100 immediately followed by 5 column volumes of PBS.
Purification procedures
1. Fill the pump tubing or syringe with binding buffer. Connect the column to the syringe (use the connector supplied) or pump tubing “drop to drop” to avoid introducing air into the column. 2. Remove the snap-off end at the column outlet. 3. Equilibrate the column with 5 column volumes of binding buffer using up to 1 ml/min (1 ml column) and up to 2.5 ml/min (5 ml column). 4. Apply the sample using a syringe fitted to the Luer connector or by pumping it onto the column. For best results, use a flow rate of 0.2 to 1 ml/min (1 ml column) and 0.5 to 2 ml/min (5 ml column) during sample application. 5. Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent. A flow rate of 1 ml/min (1 ml column) and 5 ml/min (5 ml column) is recommended for washing. 6. Elute with 5 to 10 column volumes of elution buffer. A flow rate of 1 ml/min (1 ml column) and 5 ml/min (5 ml column) is recommended for elution.
Pack size
5 × 1 mL
Dimensions
7 × 25 mm
Column volume
1 ml

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CREATIVE BIOMART®
Chromatography

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Column i.d.
7 mm
Column hardware pressure limit
5 bar (0.5 MPa)

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