

GSTrap FF Columns

Product Information

Cat#No# GST-098P

Product Overview

GSTrap FF columns are prepacked Glutathione Sepharose Fast Flow columns for fast, convenient, one-step purification of glutathione S-transferase (GST) tagged proteins.

Description

GST-tagged proteins can be purified directly from pretreated bacterial lysates using GSTrap FF. 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 4 Fast Flow, is also available as lab packages and is an excellent choice for scale-up. The columns can be operated with a syringe, peristaltic pump, or liquid chromatography system.

Characteristic

Fast and simple one-step purification of GST-tagged proteins.
Mild elution conditions preserving protein antigenicity and function.
Easy scale-up by connecting the columns in series.
Sepharose 4 Fast Flow provides good flow properties.

Applications

For the purification of GST-tagged proteins from bacterial lysates and other glutathione S-transferases or glutathione-dependent proteins and for preparation of cytoplasmic extract.

Maximum operating pressure

5 bar [0.5 MPa] (70 psi)

Sample preparation

The sample should be centrifuged and/or filtered through a 45 µm filter immediately before it is applied to the

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column. If the sample is too viscous, dilute it with binding buffer to prevent clogging the column.

Matrix

Highly cross-linked 4% agarose

Average particle size

90 µm

Ligand

Glutathione and 10-carbon linker arm

Ligand density

120 to 320 µmol glutathione/ml medium

Dynamic binding capacity

11 mg GST-tagged protein/ml medium Mr 43 000 (GSTrap FF 1 ml at 1 ml/min).

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 hour at room temperature.

CIP stability

3 to 12

Storage

4 to 30°C, 20% Ethanol

Binding buffer

PBS, pH 7.3 (140 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, pH 7.3).

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Elution buffer

50 mM Tris-HCl, 10 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.
 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 1 to 5 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 to 2 ml/min (1 ml column) and 5 to 10 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 to 2 ml/min (1 ml column) and 5 to 10 ml/min (5 ml column) is recommended for elution.
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Pack size

5 × 1 mL

Maximum flow velocity

4 ml/min and 15 ml/min for 1 ml and 5 ml columns respectively.

Dimensions

7 × 25 mm

Column volume

1 ml

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Column i.d.

7 mm

Column hardware pressure limit

5 bar (0.5 MPa)
