GE Healthcare

Instructions 28-9413-27 AB

GSTPrep FF 16/10

Introduction

GSTPrep™ FF 16/10 is a prepacked, ready to use column containing Glutathione Sepharose™ 4 Fast Flow designed for one-step purification of glutathione S-transferase (GST) tagged proteins, other glutathione S-transferases, and glutathione binding proteins.

Column data

Matrix	Glutathione Sepharose 4 Fast Flow
Bead structure	Highly cross-linked 4% agarose
Mean particle size	90 µm
Ligand	Glutathione
Ligand concentration	120–320 µmol glutathione/ml medium
Binding capacity*	= 10 mg recombinant glutathione S-transferase/ml medium (GST, M_r 26 000)
Bed volume	20 ml
Bed height	100 mm
i.d.	16 mm
Column hardware	Polypropylene
Recommended flow rate*,†	1–10 ml/min (30–300 cm/h)
Maximum flow rate*,†	10 ml/min (300 cm/h)
Maximum pressure over the packed bed during operation, Δp	0.15 MPa, 1.5 bar, 22 psi
HiPrep column hardware pressure limit‡	0.5 MPa, 5 bar, 73 psi
pH stability	pH 3–12
Storage	4°C to 30°C in 20% ethanol

* Note: The binding of GST to glutathione is depending on the flow rate and therefore can often a low flow rate increase the binding capacity. This is important during loading of sample and elution.

† Water at room temperature. Flow rate is determined by v • ≤ 10 ml/min where v = flow rate and = viscosity.

‡ Many chromatography systems are equipped with pressure gauges to measure the pressure at a particular point in the system, usually just after the pumps. The pressure measured here is the sum of the pre-column pressure, the pressure drop over the medium bed, and the post-column pressure. It is always higher than the pressure drop over the bed alone.
We recommend keeping the pressure drop over the bed below 1.5 bar. Setting the upper limit of your pressure gauge to 1.5 bar will ensure the pump shuts down before the medium is overpressured.
If necessary, post-column pressure of up to 3.5 bar can be added to the limit without exceeding the column hardware limit. To determine post-column pressure, proceed as follows:

To avoid breaking the column, the post-column pressure must never exceed 3.5 bar.

1. Connect a piece of tubing in place of the column.
2. Run the pump at the maximum flow you intend to use for chromatography. Use a buffer with the same viscosity as you intend to use for chromatography. Note the back pressure as total pressure.
3. Disconnect the tubing and run at the same flow rate used in step 2.
   Note this back pressure as pre-column pressure.
4. Calculate the post-column pressure as total pressure minus pre-column pressure.
   If the post-column pressure is higher than 3.5 bar, take steps to reduce it (shorten tubing, clear clogged tubing, or change flow restrictors) and perform steps 1–4 again until the post-column pressure is below 3.5 bar. When the post-column pressure is satisfactory, add the post-column pressure to 1.5 bar and set this as the upper pressure limit on the chromatography system.

First time use

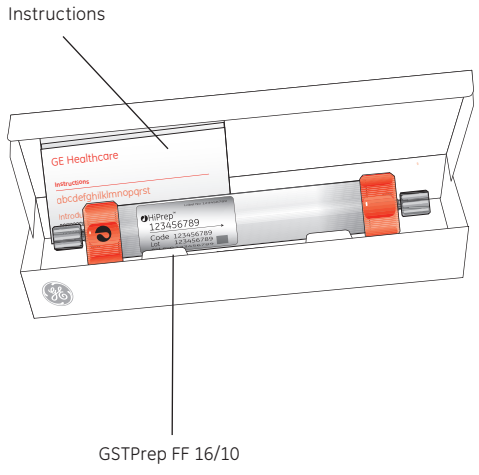
Ensure an appropriate pressure limit has been set. Equilibrate the column for first time use or after long storage by running:

100 ml binding buffer e.g. PBS, pH 7.3 (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.3 at 5 ml/min.

GSTPrep FF 16/10 column can be used directly on ÄKTAdesign™ systems without the need for any extra connectors.

Try these conditions first

Binding buffer	PBS, pH 7.3 (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.3)
Elution buffer	50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0
Flow rate	Sample loading: 1–5 ml/min (30–150 cm/h) Washing and elution: 2–10 ml/min (60–300 cm/h)



Buffers and solvent resistance

De-gas and filter all solutions through a 0.45 µm filter to increase column lifetime.

Daily use

All commonly used aqueous buffers

Cleaning

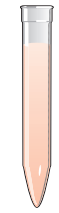
Guanidine hydrochloride, up to 6 M for 1 h at room temperature
1 M acetate pH 4.0 for 1 h at room temperature
Ethanol, up to 70%
Non-ionic detergents, e.g. Triton™ X-100, up to 1%

Avoid

Unfiltered solutions

Sample preparations

Filter the sample through 0.45 µm filter or centrifuge at 10 000 × g for 10 min. If possible dissolve the sample in binding buffer.



Delivery/storage

The column is supplied in 20% ethanol. If the column is to be stored for more than two days after use, clean the column according to the procedure described under "Cleaning in place (CIP)". Then equilibrate with at least 100 ml 20% ethanol at a flow rate of 5 ml/min

Note: HiPrep columns cannot be opened or refilled.

HiPrep

Optimization

Perform your first run according to "Try these conditions first". If the results are unsatisfactory, consider the following:

Action	Effect
Decrease flow rate	Increased binding capacity. Due to the relatively slow binding kinectics between GST and glutathione, it is important to keep the flow rate low during sample loading/elution for maximum binding capacity/elution.

The concentration of GST-tagged protein can be estimated by measuring the absorbance at 280 nm. The GST-tag can be approximated using the conversion; A_{280} = 1, which corresponds to ~ 0.5 mg/ml.

The concentration of GST-tagged protein may also be determined by standard chromogenic methods (for example Lowry, BCA, and Bradford assays). If Lowry or BCA assays are to be used, the sample must first be buffer exchanged using a HiTrap™ Desalting column, HiPrep™ 26/10 Desalting column, or dialyzed against PBS to remove glutathione, which can interfere with the protein measurement. The Bradford method can be used in the presence of glutathione.

The reuse of GSTPrep FF 16/10 depends on the nature of the sample and should only be performed with identical samples.

Cleaning-in-place (CIP)

If the medium appears to be losing binding capacity, it may be due to an accumulation of precipitate, denatured or non-specifically bound proteins.

Removal of precipitated or denatured substances:

- Wash with 40 ml of 6 M guanidine hydrochloride, immediately followed by 100 ml of PBS, pH 7.3 at a flow rate of 5 ml/min.

Removal of hydrophobically bound substances:

- Wash with 60–80 ml of 70% ethanol or 40 ml of 1% Triton X-100 immediately followed by 100 ml of PBS, pH 7.3 at a flow rate of 5 ml/min.

Note: HiPrep columns cannot be opened or refilled.

Troubleshooting

Symptom	Remedy
Low yield of eluted protein from the column	Decrease flow rate. The 10 mM recommended in this protocol should be sufficient for most applications, but exceptions may occur. Try 50 mM Tris-HCl, 20–40 mM glutathione, pH 8.0 as elution buffer to increase efficiency of eluting the protein. A low pH may limit elution from GSTPrep FF 16/10. Increasing the pH of the elution buffer to pH 8–9 may improve elution without requiring an increase in the concentration of glutathione. Including 0.1–0.2 M NaCl to the elution buffer may improve results. Non-specific hydrophobic interactions may prevent solubilization and elution of tagged proteins from GSTPrep FF 16/10. Adding a non-ionic detergent may improve results Adding 0.1% Triton or 2% N-octylglucoside can significantly improve elution of some GST-tagged proteins.
Increased back pressure	Reverse the flow direction and pump 100 ml elution buffer through the column at a flow rate of 5 ml/min at room temperature. Return to normal flow direction and run 100 ml binding buffer at a flow rate of 5 ml/min. (Try different cleaning procedures described in section "Cleaning-in-place (CIP)".
Loss of resolution and/or decreased sample recovery	Try different cleaning procedures described in section "Cleaning-in-place (CIP)".
Air in the column	Reverse the flow direction and pump 100 ml of well de-gassed binding buffer through the column at a flow rate of 5 ml/min at room temperature.

Intended use

The GSTPrep FF 16/10 is intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Ordering information

Product	No. per pack	Code No.
GSTPrep FF 16/10	1 x 20 ml	28-9365-50

Related products

GSTrap™ FF	2 x 1 ml	17-5130-02
GSTrap FF	5 x 1 ml	17-5130-01
GSTrap FF	100 × 1 ml*	17-5130-05
GSTrap FF	1 x 5 ml	17-5131-01
GSTrap FF	5 x 5 ml	17-5131-02
GSTrap FF	100 × 5 ml*	17-5131-05
Glutathione Sepharose 4 Fast Flow	25 ml	17-5132-01
Glutathione Sepharose 4 Fast Flow	100 ml	17-5132-02
Glutathione Sepharose 4 Fast Flow	500 ml	17-5132-03
HiPrep 26/10 Desalting	1 x 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 x 53 ml	17-5087-02
HiTrap Desalting	5 x 5 ml	17-1408-01
HiTrap Desalting	100 × 5 ml*	11-0003-29

* Special pack size delivered on specific customer order.

Accessories

HiTrap/HiPrep 1/16" male connector to ÄKTAdesign	8	28-4010-81
To connect columns with 1/16" connections to FPLC™ System: Union M6 female/1/16" male	5	18-3858-01

Related printed literature

GST Gene Fusion System Handbook	18-1157-58
The Recombinant Protein Purification Handbook, Principles and Methods	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography Columns and Media, Selection guide	18-1121-86
Prepacked chromatography columns for ÄKTAdesign and Ettan™ LC systems, Selection guide	28-9317-78
Glutathione Sepharose, Selection guide	28-9168-33

Further information

For more information, please refer to
www.gelifescience.com/protein-purification
www.gelifesciences.com/purification_techsupport
or refer to "GST Gene Fusion System Handbook" and "The Recombinant Protein Purification Handbook" which can be ordered, see Ordering information.

www.gelifesciences.com/protein-purification
www.gelifesciences.com/purification_techsupport

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