



HiPur Glutathione 4FF

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1. Product Description

Glutathione Beads 4FF is an affinity chromatography medium designed for the purification of glutathione S-transferase (GST)-tagged proteins produced using the pGEX series of expression vectors, other glutathione S-transferases and glutathione binding proteins. The glutathione ligand is coupled to highly cross-linked 4% agarose beads. The coupling is optimized to give high binding capacity for GST-tagged proteins and other glutathione binding proteins. **Glutathione Beads 4FF** can purify GST-tagged proteins under high flow rate. Its high flow properties make it excellent for scale-up.

HiPur Glutathione 4FF is a prepacked ready to use column. It is packed with 20ml **Glutathione Beads 4FF**. The arrow on column label indicates the recommended flow direction. It can be adapted to all kinds of chromatography system, such as ÄKTA.

Table 1. Characteristics of HiPur Glutathione 4FF

Item	Description
Size	20 ml
Column size	1.6 ×10 cm
Matrix Spherical	Highly cross-linked 6% agarose
Ligand	Reduced Glutathione
Static Binding Capacity	>10 mg GST-tagged protein /ml medium
Particle size	45-165 μm
Maximum Pressure	0.3 MPa, 3 bar
pH stability	3-12
Storage Solution	1X PBS containing 20% ethanol
Storage Temperature	2-8℃

2. Purification Procedure

2.1 Buffer Preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.22 μm or 0.45 μm filter before use.

Binding/ Wash Buffer: 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 mM GSH, pH 8.0

Note: 1–10 mM DTT can be added to Binding/ Wash Buffer or Elution Buffer.

2.2 Sample Preparation

It is recommended to filter the sample solution by passing them through a 0.22 μm or 0.45 μm filter before use.

2.3 Sample Purification

HiPur Glutathione 4FF is a prepacked, ready to use column for purification. The prepacked column provides fast, simple and easy separations in a convenient format.

1) Fill the syringe or pump tubing with distilled water. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, “drop to drop” to avoid introducing air into the column. Remove the stopper at the column outlet and connect the column to the chromatographic system.

2) Wash the column with 3-5 column volumes of distilled water.

3) Equilibrate the column with at least 5 column volumes Lysis Buffer.

4) Apply the pre-treated sample, using a Loop fitted to the connector or by pumping it onto the column.

5) Wash with Wash Buffer until the absorbance reaches the baseline or no material appears in the effluent (Generally at least 10-15 column volumes).

6) Elute with elution buffer . For one-step elution, 5 column volumes are usually enough.

2.4 Analysis

Identify the fractions containing the target protein. Use UV absorbance, SDS-PAGE, or Western Blot.





3. Cleaning-in-Place

Glutathione Beads 4FF can be reused to purify the same protein three times without regeneration. If the target GST-fusion protein is different, however, the Glutathione Beads must be regenerated using the following protocol:

Remove the precipitation or denatured protein

Wash the column with 2 column volumes 6 M guanidine hydrochloride solution. Finally wash the column with 5 column volumes of 1 × PBS (pH7.4).

Remove the hydrophobically bound protein

Wash the column with 3-4 column volumes 70% ethanol or 2 column volumes 1% Triton X-100. Finally wash the column with 5 column volumes of 1 × PBS (pH7.4).

4. Troubleshooting

Problem	Probable Cause	Solution
The yield of the purified GST fusion protein is low or undetectable.	The fusion protein forms inclusion body.	Grow bacteria at lower temperature (20-30°C), or reduce final concentration of IPTG to 0.1 mM for protein induction, or reduce the induction time. Properly dissolve and refold the inclusion body prior to the purification.
	The fusion protein does not bind to Glutathione Beads efficiently.	Increase the retention time or use batch method for purification. Incubate the clear solution (the sonicate, etc) containing GST-fusion protein with Glutathione Beads for 2 hours or longer (such as overnight) and then load the mixture onto the column.
	The fusion protein does not contain active GST.	Use mild sonication condition or other lysis method, such as lysozyme so that GST is not denatured.
	The fusion protein is degraded by protease.	Add appropriate protease inhibitors such as PMSF in the lysis solution and wash solution.
	The fusion protein is not efficiently eluted from Glutathione Beads.	Increase elution time or the concentration of reduced glutathione to 15 mM or higher in the elution buffer.
		Adjust the pH of the elution buffer to 8.0-9.0 without increasing the reduced glutathione concentration.
		Add Triton X-100 (0.1%, final concentration) or n-octyl-glucoside (2%, final concentration) or NaCl (0.1-0.2 M, final concentration) to the elution buffer.
Multiple bands observed in the eluted protein	The fusion protein is degraded by protease.	Add appropriate protease inhibitors (or inhibitor cocktails) such as PMSF in the lysis solution and wash solution.
	Some host proteins, such as chaperonins, may interact with the fusion protein.	Add DTT (5 mM, final concentration) in the wash buffer. Incubate the recombinant protein solution in chaperonin buffer (2 mM ATP, 10 mM MgSO ₄ , 50 mM Tris-HCl) at 37°C for 10 min prior to the purification.
	Over-sonication will cause some protein to bind to the fusion protein.	Use milder sonication condition or another lysis method.
	Some protein will bind to the fusion protein or beads non-specifically.	Optimizing the wash conditions. Detergents such as 1% Triton X-100, 1% Tween-20, 0.03% SDS, or 0.1% NP-40 may be used to reduce non-specific binding. Salt concentration in the wash solution can also be optimized to reduce non-specific binding.

5. Related Products

Product	Cat. No.	Size
HiSelect Glutathione 4FF	SA036C47	4.7 ml
HiPur Glutathione 4FF	SA036C20	20 ml

