Glutathione Beads 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. The characteristics of **Glutathione Beads 4FF** are summarized in Table 1 .

Table 1. Characteristics of **Glutathione Beads 4FF**

Item	Description
Matrix Spherical	highly cross-linked 4% 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 Na_2HPO4, 1.8 mM KH_2PO_4, 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 Packing of Column

Glutathione Beads 4FF is easy to pack and use, and its high flow properties make it excellent for industrial scaling-up. The method of packing the column is described below

1) Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.

2) Close the column outlet leaving the net covered with packing buffer.

3) Resuspend the beads stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.

If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

4) Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, **Glutathione Beads 4FF** is packed at a constant pressure of approximately 3 bar (0.3 MPa). If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 400 cm/h (10 cm bed height, 25℃, low viscosity buffer). If the recommended pressure or flow velocity can not be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.

5) Maintain packing flow velocity for at least 3 bed volumes. When the bed has stabilized, mark the bed height on the column and close the bottom outlet and stop the pump.

If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.

6) With the adapter inlet disconnected, push the adapter down into the column until it reaches the mark, allowing the packing solution to flush the adapter inlet. Lock the adapter in position.

7) Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.

8) Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

2.4 Sample Purification

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 snap-off end at the column outlet.

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

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

4) Apply the 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.

2.5 Analysis

Identify the fractions containing the protein. Use UV absorbance, SDS-PAGE, or western blot.

3. Cleaning-in-Place

Glutathione Beads 4FFcan 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 1XPBS (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 1XPBS (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℃), 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.

(Continued table)

Problem	Probable Cause	Solution
The yield of the purified GST fusion protein is low or undetectable.	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 degradated 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
Glutathione Beads	SA008005	5 ml
	SA008025	25 ml
	SA008100	100 ml
	SA008500	500 ml
	SA00801L	1 L
	SA00810L	10 L
GSTPur Glutathione Kit	SA008K03	3 times
	SA008K05	5 times
Glutathione Beads 4FF	SA010005	5 ml
	SA010025	25 ml
	SA010100	100 ml
	SA010500	500 ml
	SA01001L	1 L
	SA01010L	10 L
GSTCap 4FF	SA010C11	1X1 ml
	SA010C51	5X1 ml
	SA010C15	1X5 ml
	SA010C55	5X5 ml
	SA010CS	3X1 ml+1X5 ml