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Glutathione Beads Gravity Column

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1. Product Description

Glutathione Beads is an affinity chromatography medium designed for one-step purification of recombinant glutathione S-transferase (GST) fusion proteins and other glutathione binding proteins expressed in *E. coli*, insect cells and mammalian cells. The recombinant GST fusion proteins can be purified directly from pre-treated cell lysate using **Glutathione Beads**. It is the excellent choice for high performance purifications.

Glutathione Beads Gravity Column are packed with 1ml and 5ml of **Glutathione Beads**. It is easy to operate.

Table 1. Characteristics of **Glutathione Beads**

Item	Description
Matrix Spherical	4% agarose
Ligand	Reduced Glutathione
Static Binding Capacity	>20 mg GST-tagged protein(40kDa)/ml medium
Particle size	45-165 μm
Maximum Pressure	0.1 MPa, 1 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 Column Purification

For the use of **Glutathione Beads Gravity Column**, please refer to the following instructions. The amount of each solution is calculated according to the volume of the column (e.g., for 2 CV, the 1 ml size corresponds to 2 ml of solution, and the 5 ml size corresponds to 10 ml of solution). The entire purification process takes about 30 min (depending on the sample volume and the viscosity of the solution). Please refer to Figure 1 for the procedure.

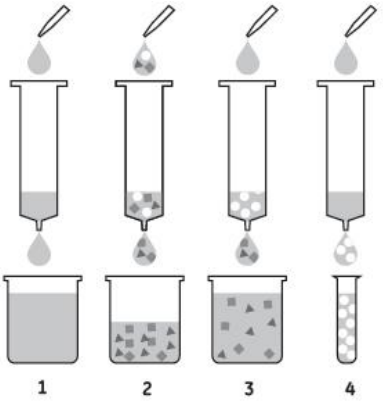

Figure 1. Schematic diagram of the protein purification process using **Glutathione Beads gravity columns**

Step 1: Column equilibration, Step 2: Sample loading, Step 3: Washing, Step 4: Elution

1) Fix **Glutathione Beads Gravity Column** on the iron stand, remove the lower and upper plugs in turn, and drain the buffer from the column. Add 5 column volumes Lysis Buffer onto the column to equilibrate the beads.
2) Apply the sample to the column and drain the flow-through. Collect the flow-through for measuring the binding efficiency to the beads, i.e. by SDS-PAGE.
3) Wash the column with 10-15 column volumes.
4) Elute the sample with 5-10 column volumes Elution Buffer and collect the eluate.
5) Equilibrate the column with 3 column volumes of Lysis Buffer , 5 column volumes of distilled water. Finally store the resin with 1×PBS containing 20% ethanol at 2℃-8℃.

2.4 Analysis

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

3. Cleaning-in-Place

Glutathione Beads 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 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°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 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 Gravity Column	SA008GC01	1 ml
	SA008GC05	5 ml
Glutathione Beads 4FF Gravity Column	SA010GC01	1 ml
	SA010GC05	5 ml