Glutathione Resin Cat. No. L00206

Technical Manual No. TM0185 **Version 07132010**

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1. Product Description

GenScript Glutathione Resin is an affinity chromatography medium designed for easy, 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 Resin. It is the excellent choice for high performance purifications. Table 1 lists the main characteristics of Glutathione Resin.

Table 1. Characteristics of Glutathione Resin

Resin volume	10 ml settled resin (20 ml 50% slurry)
Ligand	Glutathione
Dynamic binding capacity	> 6 mg horse liver GST (26 kDa)/ml settled resin
Matrix spherical	4% cross-linked agarose
Average particle size	90 µm (45-165 µm)
Storage solution	1X PBS containing 20% ethanol
Storage condition	2-8 °C
Shelf	12 months when stored unopened

2. Related Products

GenScript also provides two kits, as the derivative products of Glutathione Resin, to facilitate the expression and purification of GST fusion proteins.

GST Fusion Protein Purification Kit Cat. No. L00207

Protein Expression and Purification Kit Cat. No. L00208

Table 2. Main Components of Glutathione Resin and its derivative products

Components	L00206	L00207	L00208
Glutathione Resin	10 ml	10 ml	10 ml
Columns		5 empty columns	5 empty columns
Glutathione, reduced		5 x 0.154 g	5 x 0.154 g
Enterokinase			2 x 100 IU
Expression vector			pGS-21a
Manual	TM0185	TM0185	TM0186

3. Purification Procedure

Preparation of Cell Extract

1. Harvest cells by centrifugation at 3,000 g at 4°C for 10 min, remove and discard the supernatant.
2. Resuspend the cell pellet in 3 ml ice-cold 1×PBS buffer per 50 ml culture and centrifuge at 3,000 g at 4°C for 10 min. Remove and discard the supernatant.
3. Freeze the cell pellet at -80°C for 1 hour (This is also a convenient point to stop and one can continue the procedure later).
4. Thaw cell pellet on ice and resuspend cells in 3 ml of ice-cold 1×PBS buffer per 50 ml culture. If desired, add appropriate additives, such as non-ionic detergents (NP-40) or protease inhibitors (PMSF).
5. Disrupt cells by brief pulses of sonication on ice until the sample is no longer viscous.
6. Centrifuge at 12,000 g at 4°C for 10 min and carefully transfer the supernatant (soluble fraction) to a clean and pre-chilled tube and resuspend pellet (insoluble fraction) with 3 ml of ice-cold 1×PBS buffer per 50 ml culture.
7. Aliquot 10 µl samples from both soluble and insoluble fractions for SDS-PAGE analysis [by adding equal volume of 2X SDS Sample Buffer (125 mM Tris-HCl, pH 6.8, 4% w/v SDS, 20% glycerol, 100 mM DTT,0.02% w/v bromophenol blue), boiling for 5 min and running SDS-PAGE to determine the amount and solubility of the GST-fusion protein].

Note:

1. The binding of GST or GST-fusion protein to Glutathione Resin is not affected by 1% Triton X-100, 1% Tween-20, 1% CTAB, 10 mM DTT, 0.03% SDS, or 0.1% NP-40. These chemicals may be used to reduce non-specific binding.
2. If the target GST-fusion protein forms inclusion body (insoluble protein), the inclusion body has to be properly solubilized and refolded prior to purification.

Purification of Recombinant GST-Fusion Protein

1. Completely resuspend the Glutathione Resin by gently shaking the vial.
2. Transfer an appropriate amount of slurry to a disposable column (included in Kit L00207 and L00208). Usually 1 ml settled resin (2 ml 50% slurry) can bind more than 6 mg horse liver GST protein.
3. Wash the Glutathione Resin with 10×bed volumes of cold (4°C) 1×PBS.
4. Apply clear solution (sonicate, *etc*) containing GST-fusion protein in cold 1×PBS to the equilibrated column with the flow rate at 10-15 cm/h.
5. Add 1×PBS to wash the column just after all the protein solution get into the column, use 20×bed volumes of PBS for wash. Protease inhibitors such as PMSF are better added to wash solution to inhibit protease activity.
6. Elute the fusion protein with 10-15×bed volumes of freshly made 10 mM glutathione elution buffer (0.154 g of reduced glutathione dissolved in 50 ml of 50 mM Tris-HCl, pH 8.0.).
7. Monitor elution of the fusion protein using absorbance readings at 280 nm.
8. Aliquot 10-20 µl supernatant containing GST-fusion protein, flow-through, wash and the eluted protein, respectively, and analyze all the samples by running SDS-PAGE to confirm the presence of the target protein.
9. Pool eluted fractions containing target protein. Remove free glutathione by dialysis at 4°C against a buffer of choice or by using a G15 Sephadex desalt column.

Regeneration and Storage of Glutathione Resin

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

1. Wash the column with 2×bed volumes of 0.1 M Tris HCl + 0.5 M NaCl, pH 8.5.
2. Wash the column with 2×bed volumes of 0.1 M sodium acetate + 0.5 M NaCl, pH 4.5.
3. Re-equilibrate the column with 3-5×bed volumes of 1×PBS.
4. For long-term storage, the resin should be stored in 1×PBS containing 20% ethanol at 2 - 8°C.

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 Resin efficiently.	Use batch method for purification. Incubate clear solution (the sonicate, etc) containing GST-fusion protein with Glutathione Resin 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 Resin.	Increase elution time or increase the concentration of 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 glutathione concentration.
		Add Triton X-100 (0.1%, final concentration) or Noctylglucoside (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. Ordering Information

Glutathione Resin	Ca. No. L00206
GST Fusion Protein Purification Kit	Cat. No. L00207
Protein Expression and Purification Kit	Cat. No. L00208

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