

High-Affinity GST Resin



Technical Manual No. TM0185

Version 10252005

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I. DESCRIPTION

GenScript High-Affinity GST Resin (L00206) is designed for the rapid, single-step purification of glutathione S-transferases, glutathione-dependent proteins and recombinant derivatives of glutathione S-transferase, including glutathione S-transferase (GST) fusion proteins expressed in *E. coli*, insect cells and mammalian cells. GST fusion proteins can be purified directly from bacterial lysate using High-Affinity GST Resin. High-Affinity GST Resin (50% slurry) has a protein binding capacity of > 6 mg GST/ml packed resin.

II. SPECIFICATIONS

| | |
|-----------------------|--|
| Resin volume | 20 ml of 50% slurry |
| Ligand | Glutathione |
| Capacity | > 6 mg horse liver GST/ml packed resin |
| Matrix | Cross-linked agarose |
| Average particle size | 90 µm |

III. KEY FEATURES

- ♣ Easy to perform: simple and rapid procedure to purify GST-fusion protein.
- ♣ High capacity: > 6 mg horse liver GST/ml medium.
- ♣ Stability: no obvious decrease of the binding capacity after reusing three times.

IV. RELATED PRODUCTS

GenScript also provides two kits to facilitate the expression and purification of GST fusion proteins:

| | |
|--------|---|
| L00207 | GST Fusion Protein Purification Kit |
| L00208 | Protein Expression and Purification Kit |

| Components | L00206 | L00207 | L00208 |
|----------------------|--------|-----------------|-----------------|
| GST Resin | 20 ml | 20 ml | 20 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 |
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V. STORAGE

Store High Affinity GST Resin in 20% ethanol at 4°C, do not freeze.

VI. GST-FUSED PROTEIN PURIFICATION PROTOCOL

A. Preparation of Cell Extract

1. Harvest cells by centrifugation at 3,000 g at 4°C for 10 min, remove and discard the supernatant. Resuspend the cell pellet in 3 mL ice-cold PBS Buffer per 50 mL culture and centrifuge at 3,000 g at 4°C for 10 min. Remove and discard the supernatant.
2. 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).
3. Thaw cell pellet on ice and re-suspend cells in 3 mL of ice-cold PBS Buffer per 50 mL culture. If desired, add appropriate additives, such as non-ionic detergents (NP-40) or protease inhibitors (PMSF).
4. Break the cells by brief pulses of sonication on ice until the sample is no longer viscous.
5. 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 PBS Buffer per 50 mL of *E. coli* culture.
6. Aliquot 10 µL samples from both Soluble and Insoluble Fractions for SDS-PAGE Analysis (*by adding equal volume of 2 x SDS sample loading buffer, boil for 5 min and run SDS-PAGE to determine the amount and solubility of the GST-fusion protein*).

Note: a. The binding of GST or GST-fusion protein to High Affinity GST 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.

b. If the target GST-fusion protein forms inclusion body (insoluble protein), inclusion body has to be properly solubilized and refolded prior to purification).

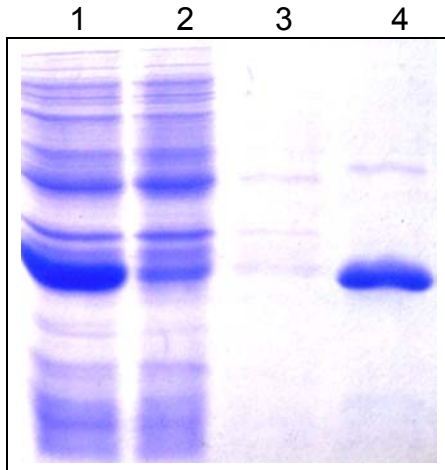
B. Purification of Recombinant GST-Fusion Protein

1. Shake gently the bottle containing the High-Affinity GST Resin until all the resin is completely in suspension.
2. Transfer an appropriate amount of resin (50% slurry) to a disposable column (included in Kit L00207 and L00208) using a pipet. Usually 1 ml of resin (from 2 ml of 50% slurry) can bind more than 6 mg of GST protein.
3. Wash the GST resin with 10 bed volumes of cold (4°C) PBS.
4. Apply clear solution (sonicate, etc) containing GST-fusion protein in PBS to the equilibrated column with the flow rate at 10-15 cm/h.
5. Add 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 the 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 of 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. An example was shown in Figure 1.
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.

C. Regeneration and Storage of High Affinity GST Resin

GenScript High-Affinity GST Resin can be reused to purify the same protein three times without regeneration. If the target GST-fusion protein is different, however, High-Affinity GST 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 1X PBS.
4. For long-term storage, the resin should be stored in 20% ethanol at 4 °C.



| | |
|---|---------------|
| 1 | Total protein |
| 2 | Flow through |
| 3 | Wash |
| 4 | Elute |

Fig.1. Purification profile using High-Affinity GST Resin

VII. Troubleshooting

The table below is guideline for troubleshooting.

| Problem | Probable Cause | Solution |
|---|--|---|
| The yield of the purified fusion protein is low or undetectable | The fusion protein forms inclusion body. | Grow bacteria at low 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 GST Resin efficiently. | Use batch method for purification. Incubate clear solution (sonicate, etc) containing GST-fusion protein with GST 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 High-Affinity GST 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 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. |

VIII. ORDER INFORMATION

High Affinity GST Resin Catalog Number: L00206

For Research Use Only.

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