INSTRUCTIONS

Pierce® Glutathione Agarose

16100 16101 16102

2248.1

Number	Description
16100	**Pierce Glutathione Agarose,** 10mL settled resin
16101	**Pierce Glutathione Agarose,** 100mL settled resin
16102	**Pierce Glutathione Agarose,** 500mL settled resin
	Binding Capacity: ≥ 40mg of purified recombinant glutathione S-transferase (GST) per milliliter of settled resin
	Resin: Crosslinked 6% agarose
	Supplied: 50% slurry in 0.05% sodium azide solution
	Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Glutathione Agarose is used for purifying GST-fusion proteins from cellular lysates. Glutathione is linked by its central sulfhydryl with a 12-atom spacer, which minimizes steric hindrance. Purification of GST-fusion proteins using glutathione agarose beads is well documented[1,2] and provides an easy-to-use, one-step, high purity affinity purification. The bound GST-fusion proteins are eluted using a buffer containing reduced glutathione, or the fusion protein can be cleaved at the GST tag using thrombin, HRV 3C protease, or Thermo Scientific Factor Xa (Product No. 32520).

Important Product Information

- Protein yield and purity are dependent upon the expression level, conformation and solubility characteristics of the recombinant fusion protein. Therefore, it is important to optimize these parameters before attempting a large-scale purification. For best results, perform a small-scale test to estimate the expression level and determine the solubility of each GST-tagged protein.
- The stated capacity of the glutathione resin is measured under saturating conditions. In a practical setting, the amount of resin to use with a given quantity of crude protein lysate is dependent upon the expression level of the GST-fusion protein and binding is influenced by factors present in the lysate as well as the lysis buffer. As a general guideline, 50-200mg of total protein lysate can be loaded onto each milliliter of resin.
- Optimization of the lysis procedure is critical for maximizing protein yield. Some methods for protein extraction include using commercially available detergent-based reagents, such as Thermo Scientific B-PER Bacterial Protein Extraction Reagent with Enzymes (Product No. 90078), and mechanical methods, such as freeze/thaw cycles, sonication or French press. Add protease inhibitors, such as Thermo Scientific Halt Protease Inhibitor Cocktail (Product No. 87786), to protect proteins from degradation.

Additional Materials Required

Purification Buffers

- Equilibration/Wash Buffer: 50mM Tris, 150mM NaCl, pH 8.0.
- Elution Buffer: 50mM Tris, 150mM NaCl, pH 8.0 containing 10mM reduced glutathione.

  Note: Adding glutathione alters the buffer's pH. Adjust the Elution Buffer's final pH to 8.0 with NaOH before use.

Buffers for Regeneration of Glutathione Resin (optional)

- Regeneration Buffer #1: 0.1M Tris containing 0.5M NaCl and 0.1% SDS, pH 8.5
- Regeneration Buffer #2: 0.1M sodium acetate containing 0.5M NaCl and 0.1% SDS, pH 4.5

Procedure for Purification of GST-Tagged Proteins by Batch Method

Note: Purification conditions can be scaled as needed. The procedure may be performed at room temperature or at 4°C.

1. Add an appropriate amount of Glutathione Agarose to a tube. Centrifuge tube for 2 minutes at 700 × *g* and carefully remove and discard the supernatant.
2. Add 10 resin-bed volumes of Equilibration/Wash Buffer and mix until the resin is fully suspended.
3. Centrifuge tube for 2 minutes at 700 × *g* and carefully remove and discard buffer.
4. For best results, prepare sample by mixing the protein extract with Equilibration/Wash Buffer so the total volume equals at least two resin-bed volumes. Other ratios may be used but need to be determined empirically.

   Note: For larger sample volumes, several applications may be performed. Do not exceed the column's binding capacity.
5. Add the prepared protein extract to the equilibrated resin and mix on an end-over-end rotator for 30-60 minutes at room temperature or 4°C.
6. Centrifuge the tube for 2 minutes at 700 × *g*. If desired, save supernatant for downstream analysis.
7. Wash the resin with 5-10 resin-bed volumes of Equilibration/Wash Buffer. Centrifuge the tube for 2 minutes at 700 × *g* and remove supernatant. If desired, save supernatant for downstream analysis.
8. Repeat wash step and monitor supernatant by measuring its absorbance at 280nm until baseline is reached.
9. Elute bound GST-tagged protein using one resin-bed volume of Elution Buffer. Centrifuge tube for 2 minutes at 700 × *g*. Carefully remove and save the supernatant. Repeat this step twice, saving each supernatant fraction in a separate tube.
10. Monitor protein elution by measuring the absorbance of the fractions at 280nm or by Thermo Scientific Coomassie Plus (Bradford) Assay Reagent (Product No. 23238) or Pierce 660 nm Protein Assay (Product No. 22660). The eluted protein can be directly analyzed by SDS-PAGE.

    Note: To remove glutathione for downstream applications use gel filtration (e.g., Thermo Scientific Zeba Spin Desalting Columns) or dialysis (e.g., Thermo Scientific Slide-A-Lyzer Dialysis Cassettes).

Procedure for Purification of GST-tagged Proteins using a Gravity-flow Column

Note: Purification conditions can be scaled as desired. Perform the procedure at room temperature or at 4°C.

1. Pack column with an appropriate amount of Glutathione Agarose. Allow storage buffer to drain from resin by gravity flow.
2. For best results, prepare sample by mixing protein extract with Equilibration/Wash Buffer so the total volume equals at least two resin-bed volumes. Other ratios may be used but need to be determined empirically.
3. Equilibrate column with 10 resin-bed volumes of Equilibration/Wash Buffer. Using a flow rate of 0.5mL/minute, allow buffer to drain from resin.
4. Add the prepared protein extract to the resin. Collect the flow-through in a tube. If desired, re-apply the flow-through once to maximize binding.
5. Wash resin with 5-10 resin-bed volumes of Equilibration/Wash Buffer and collect the flow-through. Repeat this step using a new collection tube until the absorbance of the flow-through fraction at 280nm approaches baseline.

6. Elute GST-tagged protein from the resin with two resin-bed volumes of Elution Buffer. Repeat this step twice, collecting each fraction in a separate tube.
7. Monitor protein elution by measuring the absorbance of the fractions at 280nm or by Coomassie Plus (Bradford) Assay Reagent (Product No. 23238) or Pierce 660nm Protein Assay (Product No. 22660). The eluted protein can be directly analyzed by SDS-PAGE.

   Note: To remove glutathione for downstream applications use gel filtration (e.g., Zeba™ Spin Desalting Columns) or dialysis (e.g., Slide-A-Lyzer® Dialysis Cassettes).

Procedure for Glutathione Agarose Regeneration (optional)

The Glutathione Agarose may be used at least five times without affecting protein yield or purity. Between each use, perform the procedure described below to remove residual glutathione and any nonspecifically adsorbed protein. To prevent cross-contamination of samples, designate a given column to one specific fusion protein.

1. Apply 5 resin-bed volumes of Regeneration Buffer #1.
2. Apply 5 resin-bed volumes of ultrapure water.
3. Apply 5 resin-bed volumes of Regeneration Buffer #2.
4. Apply 5 resin-bed volumes of ultrapure water.
5. Wash the column with 5mL of 0.05% sodium azide (in water). Cap bottom and top of column. Store at 4°C.

Troubleshooting

Problem	Possible Cause	Solution
Low protein yield	Poor protein expression	Optimize expression conditions
	Fusion protein forms inclusion bodies	Alter bacterial growth conditions (e.g., decrease temperature, modify induction conditions)
	Insufficient extraction	Optimize cell lysis protocol
	Fusion protein does not bind to the column	Fusion partner may have altered the conformation of GST, thereby reducing its affinity: Add 5mM DTT to lysis buffer before extraction, which can significantly increase binding of some GST-fusion proteins to the immobilized glutathione
Poor protein purity	Insufficient washing	Increase the number of washes with Wash Buffer. Alternatively, add detergent or additional salt to the Equilibration/Wash Buffer to increase the stringency
	Fusion protein has interaction(s) with other bacterial proteins	Add 5mM DTT to lysis buffer before extraction to help reduce nonspecific interactions
Slow column flow	Column is overloaded	Apply less protein extract onto the column and make sure the extract is not too viscous or highly particulate

Additional Information

A. Visit the website for additional information relating to this product including the following:

- Tech Tip # 43: Protein stability and storage
- Tech Tip # 40: Convert between times gravity (× *g*) and centrifuge rotor speed (RPM)
- Tech Tip # 6: Extinction coefficients guide

Related Thermo Scientific Products

16103, 16104, 16105	**Pierce Glutathione Spin Columns,** 0.2mL, 1mL, 3mL
16106, 16107, 16108	**Pierce GST Spin Purification Kits,** containing 0.2mL, 1mL, or 3mL spin columns
16109, 16110	**Pierce Glutathione Chromatography Cartridges,** 5 × 1mL; 2 × 5mL
16111	**Pierce Glutathione Spin Plates,** 2/pkg
88270	**Pierce High Capacity Endotoxin Removal Gel,** 10mL
88282	**Pierce LAL Chromogenic Endotoxin Quantitation Kit**
88221	**HisPur Ni-NTA Resin,** 10mL
89964	**HisPur Cobalt Resin,** 10mL
90078, 90079	**B-PER Bacterial Protein Extraction Reagent with Enzymes,** 250mL or 500mL
90084, 78248	**B-PER Bacterial Protein Extraction Reagent,** 250mL or 500mL
87786	**Halt Protease Inhibitor Cocktail (100X)**
78259	**Glutathione (reduced),** 5 x 184mg
21516	**Pierce GST Protein Interaction Pull-down Kit**
15140	**Pierce Glutathione Coated Plates**, 5 plates
MA4-004	**Anti-Glutathione S-Transferase Antibody,** 0.1mg
32520	**Factor Xa**, 250µg
20291	**DTT, No Weigh™ Format,** 7.7mg × 48

Cited References

1. Frangioni, J.V. and Neel, B.G. (1993). Solubilization and purification of enzymatically active glutathione s-transferase (pGEX) fusion proteins. *Anal Biochem* **210**:179-87.
2. Simons, P.C. and VanderJagt, D.L. (1977). Purification of glutathione S-transferases for human liver by glutathione-affinity chromatography. *Anal Biochem* **82**:334-41.

General References

1. Janknecht, R., *et al*. (1991). Rapid and efficient purification of native histidine-tagged protein expressed by recombinant vaccinia virus. *Proc Natl Acad Sci USA*. **88:**8972-6.
2. Riggs, P., in Ausubsel. F.M., *et al*. (eds). (1990). *Curr Protoc Mol Biol* 16.4.1-16.6.14.
3. Smith, D.B. and Johnson, K.S. (1988). Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. *Gene* **7**:31-40.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.