

Pierce[®] Glutathione Spin Columns

16103 16104 16105

2249.2

Number	Description
16103	Pierce Glutathione Spin Columns, 0.2mL resin bed, 25 columns
16104	Pierce Glutathione Spin Columns, 1mL resin bed, 5 columns
16105	Pierce Glutathione Spin Columns, 3mL resin bed, 5 columns

Binding Capacity: \geq 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

Thermo Scientific Pierce Glutathione Spin Columns provide a fast, easy-to-use format to purify GST-fusion proteins from cellular lysates. The glutathione is immobilized through its central sulfhydryl onto 6% crosslinked agarose resin. Purification of GST-fusion proteins using glutathione agarose beads is well documented^{1,2} and provides a 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 pH 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 Spin Purification of GST-Tagged Proteins

Note: The total volume of the 0.2, 1 and 3mL columns are 0.8, 8 and 22mL, respectively. If a sample volume is greater than the column, perform multiple applications and centrifugations until the entire sample has been processed. Be careful not to exceed the resin's binding capacity. The Pierce Glutathione Spin Columns also may be used for gravity-flow purifications.

1. Equilibrate column(s) to working temperature. Perform purifications at room temperature or at 4°C.
2. 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.

3. Remove the bottom tab from the Pierce Glutathione Spin Column by gently twisting. Place column into a centrifuge tube.

Note: Use 1.5, 15 or 50mL centrifuge tubes for the 0.2, 1 and 3mL spin columns, respectively.

4. Centrifuge column at $700 \times g$ for 2 minutes to remove storage buffer.
5. Equilibrate column with two resin-bed volumes of Equilibration/Wash Buffer. Allow buffer to enter the resin bed.
6. Centrifuge column at $700 \times g$ for 2 minutes to remove buffer.
7. Add the prepared protein extract to the column and allow it to enter the resin bed.

Note: For maximal binding, the sample can be incubated for 30-60 minutes at room temperature or 4°C on an end-over-end rocking platform.

8. Centrifuge column at $700 \times g$ for 2 minutes and collect the flow-through in a centrifuge tube. If desired, save flow-through fraction for downstream analysis.
9. Wash resin with two resin-bed volumes of Equilibration/Wash Buffer. Centrifuge at $700 \times g$ for 2 minutes and collect fraction in a centrifuge tube. Repeat this step two more times collecting each fraction in a separate centrifuge tube. Monitor the absorbance of the washes at 280nm and perform additional washes if necessary until the absorbance approaches baseline.
10. Elute GST-tagged protein from the resin by adding one resin-bed volume of Elution Buffer. Centrifuge at $700 \times g$ for 2 minutes. Repeat this step two more times, collecting each fraction in a separate tube.

Note: If performing gravity-flow add two resin-bed volumes of Elution Buffer to achieve proper flow characteristics. Repeat this step two more times, collecting each fraction in a separate tube.

11. 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 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., Thermo Scientific Zeba Spin Desalting Columns) or dialysis (e.g., Thermo Scientific 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 as 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 formed inclusion bodies	Alter bacterial growth conditions (e.g., decrease temperature, modify induction conditions)
	Insufficient extraction	Optimize cell lysis protocol
	Fusion protein did 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 had interaction(s) with other bacterial proteins	Add 5mM DTT to lysis buffer before extraction to help reduce nonspecific interactions
Slow column flow	Column was overloaded	Apply less protein extract onto the column and make sure the extract is not too viscous or highly particulate

Additional Information Available on Our Website

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

Related Thermo Scientific Products

16106, 16107, 16108	GST Spin Purification Kits , containing 0.2mL, 1mL, or 3mL spin columns
16100, 16101, 16102	Pierce Glutathione Agarose , 10mL 100mL, or 500mL
16109, 16110	Pierce Glutathione Chromatography Cartridges , 5 \times 1mL; 2 \times 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 × 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
23238	Coomassie Plus™ (Bradford) Assay Reagent, 300mL
22660	Pierce 660nm Protein Assay Reagent, 750mL
89882	Zeba™ Spin Desalting Columns
87717	Slide-A-Lyzer® G2 Dialysis Cassettes
20291	DTT, No-Weigh™ Format, 7.7mg x 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 Ausubel, 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.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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

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