Pierce™ Glutathione Spin Columns

Catalog Numbers 16103, 16104, and 16105

Doc. Part No. 2162249 Pub. No. MAN0011720 Rev. B

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

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 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 (Cat. No. 32520).

Contents and storage

Item	Catalog Number	Storage
Pierce™ Glutathione Spin Columns, 0.2 mL resin bed, 25 columns	16103	Store at 4°C
Pierce™ Glutathione Spin Columns, 1 mL resin bed, 5 columns	16104	
Pierce™ Glutathione Spin Columns, 3 mL resin bed, 5 columns	16105	
Binding Capacity: ≥40 mg of purified recombinant glutathione S-transferase (GST) per milliliter of settled resin Resin: Crosslinked 6% agarose supplied as 50% slurry in 0.05% sodium azide solution		

Required materials not supplied

Purification buffers

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

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

(Optional) Buffers for regeneration of glutathione resin

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

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, 50-200 mg 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.

Procedure for spin purification of GST-Tagged proteins

Note: The total volume of the 0.2-, 1-, and 3-mL columns are 0.8 mL, 8 mL, and 22 mL, 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 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 (SAVE bottom closure for later use). Place column into a centrifuge tube.

   Note: Use 1.5-, 15-, or 50-mL centrifuge tubes for the 0.2 mL, 1 mL, and 3 mL spin columns, respectively.
4. Centrifuge column at 700 x *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 x *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 × *g* for 2 minutes and collect the flowthrough in a centrifuge tube. If desired, save flowthrough fraction for downstream analysis.
9. Wash resin with two resin-bed volumes of Equilibration/Wash Buffer. Centrifuge at 700 x *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 280 nm 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 x *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 280 nm or by Thermo Scientific™ Coomassie Plus™ (Bradford)™ Assay Reagent (Cat. No. 23238) or Pierce™ 660 nm Protein Assay (Cat. No. 22660). The eluted protein can be directly analyzed by SDS-PAGE.

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

(Optional) Procedure for glutathione agarose regeneration

The Glutathione Agarose may be used at least 5 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 5 mL of 0.05% sodium azide (in water). Cap the bottom (invert snap-off closure and apply to the column bottom with gentle pressure) 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 (for example, 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 5 mM 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 5 mM 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.

Related products

Product	Cat. No.
GST Spin Purification Kits, containing 0.2 mL, 1 mL, or 3 mL spin columns	16106, 16107, 16108
Pierce™ Glutathione Agarose, 10 mL 100 mL, or 500 mL	16100, 16101, 16102
Pierce™ Glutathione Chromatography Cartridges, 5 × 1 mL; 2 × 5 mL	16109, 16110
Pierce™ Glutathione Spin Plates, 2/pkg	16111
Pierce™ High Capacity Endotoxin Removal Gel, 10 mL	88270
Pierce™ LAL Chromogenic Endotoxin Quantitation Kit	88282
HisPur™ Ni-NTA Resin, 10 mL	88221
HisPur™ Cobalt Resin, 10 mL	89964
B-PER™ Bacterial Protein Extraction Reagent with Enzymes, 250 mL or 500 mL	90078, 90079
B-PER™ Bacterial Protein Extraction Reagent, 250 mL or 500 mL	90084, 78248
Halt™ Protease Inhibitor Cocktail (100X)	87786
Glutathione (reduced), 5 × 184 mg	78259
Pierce™ GST Protein Interaction Pull-down Kit	21516
Pierce™ Glutathione Coated Plates, 5 plates	15140
Anti-Glutathione S-Transferase Antibody, 0.1 mg	MA4-004
Factor Xa, 250 µg	32520
Coomassie Plus™ (Bradford)™ Assay Reagent, 300 mL	23238
Pierce™ 660 nm Protein Assay Reagent, 750 mL	22660
Zeba™ Spin Desalting Columns	89882
Slide-A-Lyzer™ G2 Dialysis Cassettes	87717
DTT, No-Weigh™ Format, 7.7 mg x 48	20291

General references

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- 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.
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- 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.
- 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.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0011720 B

Revision	Date	Description
B	10 May 2024	Removing bottom caps to correspond with product change.
A.0	17 October 2015	New document for Pierce™ Glutathione Spin Columns.