INSTRUCTIONS

Pierce™ GST Spin Purification Kit

Pub. No. MAN0011721
Rev. B
Doc. Part No. 2162250

16106 16107 16108

Number	Description
16106	**Pierce GST Spin Purification Kit, 0.2mL**
	Kit Contents:
	Pierce Glutathione Spin Columns, 0.2mL resin bed, 25 each
	Equilibration/Wash Buffer, 250mL, 125mM Tris, 150mM sodium chloride, pH 8.0
	Glutathione, 184mg
	Collection Tubes, 80 each
16107	**Pierce GST Spin Purification Kit, 1mL**
	Kit Contents:
	Pierce Glutathione Spin Columns, 1mL resin bed, 5 each
	Equilibration/Wash Buffer, 250mL, 125mM Tris, 150mM sodium chloride, pH 8.0
	Glutathione, 184mg
16108	**Pierce GST Spin Purification Kit, 3mL**
	Kit Contents:
	Pierce Glutathione Spin Columns, 3mL resin bed, 5 each
	Equilibration/Wash Buffer, 250mL, 125mM Tris, 150mM sodium chloride, pH 8.0
	Glutathione, 184mg

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 GST Spin Purification Kits contain Pierce Glutathione Spin Columns and buffer components to provide a fast, easy-to-use format for purifying 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

Buffers for Regeneration of Glutathione Resin (optional)

- Regeneration Buffer #1: 0.1M Tris containing 0.5 M 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

Material Preparation

The Elution Buffer contains glutathione, which is prone to oxidation during prolonged storage. Reconstituted 10X Glutathione should be stored at -20°C until ready to use.

10X Glutathione	Add 6mL Equilibration/Wash Buffer to vial of Glutathione provided. Recap vial and mix until the glutathione is completely dissolved. Store 10X stock at -20°C up to 18 months.
Elution Buffer	Dilute 10X Glutathione stock solution 10-fold with Equilibration/Wash Buffer (example: add 1mL 10X Glutathione to 9mL Equilibration/Wash Buffer). For best results, make fresh Elution Buffer for each use. Alternatively, the Elution Buffer may be stored up to 2 weeks at 4°C with no functional loss.

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 protein extract with Equilibration/Wash Buffer so that 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 as long as the column capacity is not exceeded.
3. Remove bottom closure 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 50mL centrifuge tubes for the 0.2, 1 and 3mL spin columns, respectively.
4. Centrifuge column at 700 × *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 × *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 flow-through fraction for downstream analysis.
9. Wash resin with two resin-bed volumes of Equilibration/Wash Buffer. Centrifuge at 700 × *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.

   Note: If desired, perform additional washes. Monitor washes by measuring their absorbance at 280nm.
10. Elute GST-tagged protein from the resin by adding one resin-bed volume of Elution Buffer. Centrifuge at 700 × *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 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.

Wash the column with 5mL of 0.05% sodium azide (in water). Reseal the column by inverting the original snap-off closure, and with a slight twisting motion, press it firmly to the bottom tip of the column, then cap the top of the 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.

Related Thermo Scientific Products

16103, 16104, 16105	**Pierce Glutathione Spin Columns,** 0.2mL, 1mL, or 3mL
16100, 16101, 16102	**Pierce Glutathione Agarose,** 10mL, 100mL, or 500mL
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 × 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
22660	**Pierce 660nm Protein Assay Reagent,** 750mL
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.

Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition. The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0011721 B

Revision	Date	Description
B	31 July 2024	Correcting spin column usage.
A	17 October 2015	New document for Pierce™ GST Spin Purification Kit.