INSTRUCTIONS

Pierce® Glutathione Chromatography Cartridge

16109 16110

2251.1

Number	Description
16109	**Pierce Glutathione Chromatography Cartridge,** 5 × 1mL
16110	**Pierce Glutathione Chromatography Cartridge,** 2 × 5mL

Each product contains an accessory pack (1 female Luer-Lok® Adapter, 1 connector fitting, 1 column plug and bottom caps).

Binding Capacity: ≥ 40mg of purified recombinant glutathione S-transferase (GST) per milliliter of settled resin

Resin: Crosslinked 6% agarose supplied in 0.05% sodium azide solution.

Storage: Upon receipt store at 4-8°C. Product is shipped at ambient temperature. Do not freeze.

Introduction

The Thermo Scientific Pierce Glutathione Chromatography Cartridges are convenient, ready-to-use pre-packed devices for the purification of GST-fusion proteins from cellular lysates. Glutathione is linked to the resin support through its central sulfhydryl using 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).

Thermo Scientific Cartridges are compatible with the major automated liquid-chromatography systems or manual syringe processing (see Table 1). The cartridges attach directly to ÄKTA™ or FPLC Systems without additional connectors. The included accessory pack readily adapts cartridges for use with Luer-Lok Syringe Fittings or 1/16" tubing. These cartridges enable fast, easy and reproducible chromatographic separations and can be regenerated for multiple uses.

Table 1. Properties of the Thermo Scientific Pierce Glutathione Chromatography Cartridges.

Support	Crosslinked 6% agarose
Ligand	Glutathione linked by 12-atom spacer
Binding Capacity	≥ 25mg of recombinant glutathione S-transferase (GST) per milliliter of settled resin
Cartridge Dimensions	0.7 × 2.7cm (1mL column); 1.3 × 3.8cm (5mL column)
Particle Size	45-165µm
Void Volume	0.32mL (1mL column); 1.5mL (5mL column)
Recommended Flow Rates	Sample Application:
	0.2-1mL/min (1mL cartridge)
	0.5-2mL/min (5mL cartridge)
	Wash and Elution:
	1-2mL/min (1mL cartridge)
	1-5mL/min (5mL cartridge)
pH Stability	4-13
Maximum Operating Pressure	0.3MPa, 43.5psi or 3 bar
Cartridge Material	Polypropylene
Frit	Polyethylene, 10µm pore size
Accessory Pack	Luer-Lok Adapter to 10-32 male, plug for 10-32 coned port, cap 1/16 male
	Finger-tight 10-32 connector fitting for 1/16" OD tubing

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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.
- Best results are obtained by keeping the overall purification time as short as possible; however, the slower binding kinetics between GST and glutathione require a lower flow rate during the sample application step to maximize binding capacity.
- For liquid-chromatography applications use highly pure buffer components and water. For best results, filter buffers through a 0.45µm filter and degas before use.

Additional Materials Required

- Suitable liquid chromatography system (LC procedure) with 1/16" tubing or syringes
- Additional connectors and fittings are required to attach to the Bio-Rad BioLogic™ System.

Purification Buffers

- Binding 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 Cartridge (optional)

- Regeneration Reagent 1: 0.1M Tris containing 0.5M NaCl and 0.1% SDS; pH 8.5 (five column volumes) or 6M guanidine hydrochloride (two column volumes, slow flow rate)
- Regeneration Reagent 2: 0.1M sodium acetate containing 0.5M NaCl and 0.1% SDS; pH 4.5 (five column volumes) or 1% Triton X-100 (two column volumes, slow flow rate) or 70% ethanol (3-4 column volumes, slow flow rate)

  Note: SDS forms a precipitate at 4°C; use at ambient temperature

Procedure for Purifying GST-tagged Proteins Using a Liquid-Chromatography System

Note: For syringe application, 30 drops per minute is equivalent to a flow rate of 1mL per minute.

1. Equilibrate the cartridge and all buffers to working temperature. Perform purifications at room temperature or at 4°C. Ensure that all solutions are degassed.
2. Prepare the LC system by filling tubing with buffer. Remove top plug from cartridge and carefully snap off the end-tab (do not twist). To avoid introducing air into the system, let a few drops of buffer flow from tubing into cartridge top then connect cartridge top to the tubing; allow a few drops to emerge from the cartridge before connecting to the LC inlet port.
3. Equilibrate the cartridge with 5-10 column volumes of Binding Buffer at a flow rate of 1-2mL/minute for the 1mL cartridge or 1-5mL/minute for the 5mL cartridge.
4. Mix the sample 1:1 with Binding Buffer to adjust the ionic strength and pH. Alternatively, buffer-exchange the sample against the Binding Buffer. If the sample contains insoluble matter, centrifuge or filter (0.45µm) the sample immediately before use. Apply a volume that does not exceed column capacity.
5. Apply the clarified sample to the cartridge. For maximum binding, apply at a flow rate of 0.2-1mL/minute for the 1mL cartridge and 0.5-2mL/minute for the 5mL cartridge. Optional: Retain flow-through fractions for downstream analysis.

6. Wash the resin with 10-15 column volumes of Binding Buffer or until the absorbance approaches baseline.
7. Elute bound GST-tagged protein with approximately 5-10 column volumes of Elution Buffer and collect fractions. Elute using a one-step or linear gradient.
8. Monitor protein elution by measuring the absorbance of the fractions at 280nm or by Thermo Scientific Pierce 660nm Protein Assay (Product No. 22660) or Coomassie Plus (Bradford) Assay Reagent (Product No. 23238). The eluted protein can be directly analyzed by SDS-PAGE.

   Note: To remove glutathione for downstream applications use gel filtration (e.g. Thermo Scientific Pierce Desalting Chromatography Cartridges, Zeba Spin Desalting Columns or Slide-A-Lyzer Dialysis Cassettes).
9. For storage, wash the cartridge with five column volumes of water and store in 0.05% sodium azide. Attach supplied bottom cap followed by the top plug. Store the cartridge at 4°C.

Procedure for Cartridge Regeneration

The Glutathione Cartridge can be used multiple times without affecting protein yield or purity. An accumulation of denatured, precipitated, or nonspecifically bound substances may cause the cartridge to lose binding capacity. The cartridge may be regenerated with the procedure listed below. To prevent cross contamination of samples, designate a given cartridge to one specific fusion protein.

1. Wash cartridge with Regeneration Reagent 1, followed by five column volumes of ultrapure water.
2. Wash cartridge with Regeneration Reagent 2, followed by five column volumes of ultrapure water.
3. For storage: Wash the cartridge with five column volumes of 0.05% sodium azide (in ultrapure water). Cap bottom and top of column and store at 4°C.
4. Before reuse: Equilibrate the cartridge with 5-10 column volumes of Binding Buffer.

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
	Flow rate is too fast	Decrease the flow rate during sample application
Poor protein purity	Insufficient washing	Increase the number of washes with Binding Buffer or add detergent (0.05% NP-40) or increase salt concentration in the Binding 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 sample to the column or decrease the flow rate
High back pressure (exceeds 0.3MPa)	Cell debris clogging the cartridge	Dilute the sample with Binding Buffer. For highly particulate extracts, clarify the sample by filtration (0.45µm) or centrifugation

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 # 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
16100, 16101, 16102	**Pierce Glutathione Agarose**, 10mL, 100mL, 500mL settled resin
88270	**Pierce High Capacity Endotoxin Removal Gel,** 10mL
88282	**Pierce LAL Chromogenic Endotoxin Quantitation Kit**
90093	**HisPur Cobalt Chromatography Cartridges, 1mL,** 5/pkg
90094	**HiPur Cobalt Chromatography Cartridges, 5mL,** 2/pkg
90098	**HisPur Ni-NTA Chromatography Cartridges, 1mL,** 5/pkg
90099	**HisPur Ni-NTA Chromatography Cartridges, 5mL,** 2/pkg
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
15140	**Pierce Glutathione Coated Plates**, 5 plates
MA4-004	**Anti-Glutathione S-Transferase Antibody,** 0.1mg
32520	**Factor Xa**, 250µg
89934	**Desalting Chromatography Cartridges**, 5 × 5mL
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 from 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.

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