INSTRUCTIONS

Pierce™ Glutathione Superflow Agarose

25236 25237 25238 25239

2411.0

Number	Description
25236	**Pierce Glutathione Superflow Agarose,** 10mL settled resin
25237	**Pierce Glutathione Superflow Agarose,** 50mL settled resin
25238	**Pierce Glutathione Superflow Agarose,** 250mL settled resin
25239	**Pierce Glutathione Superflow Agarose,** 1000mL settled resin

Binding Capacity: ≥ 30mg/mL of settled resin for a recombinant glutathione s-transferase (GST) (molecular weight 26kDa)

Resin: Highly crosslinked 6% Superflow Agarose

Supplied: 50% slurry in 0.05% sodium azide

Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Glutathione Superflow Agarose enables high-purity purification of GST fusion proteins. The glutathione is immobilized by its central sulfhydryl group via a 12-atom spacer arm to a highly crosslinked 6% Superflow Agarose bead. The agarose is compatible with a wide range of chemicals and pH values and is stable to multiple reuses. The resin supports a high dynamic-binding capacity across a wide range of flow rates making it ideal for large-scale FPLC purifications.

Table 1. Properties of Thermo Scientific Pierce Glutathione Superflow Agarose.

Support :	Superflow 6 Resin, 6% highly crosslinked agarose
Bead Size :	60-160µm
Recommended Liner Flow Rate:	≤ 150cm/hr (binding, wash, elution)
Maximum Liner Flow Rate†:	1200cm/hr
Capacity:	Typical Static Capacity: ~30mg GST/mL resin
	Typical Dynamic Capacity*: ~10mg GST/mL resin
Chemical Compatibility:	1M acetic acid, pH 2; 1% SDS, pH 7; 6M guanidine•HCl; 70% EtOH: ≥ 1 week at 37°C
	8M urea; 0.1M HCl, pH 1; 0.1M NaOH (≥ 2 hours at 22°C)
pH Limits:	pH 3-9 (≥ 1 week at 4°C)
	pH 2-3 or 9-12 (≥ 2 hours at 22°C)
Storage Solution:	0.05% NaN_3
Reuse:	≥ 25 times

†Max linear flow rate conditions
Column dimensions (w × h): 13mm x 38mm (5mL resin)
Ultrapure water at room temperature
Linear flow rate = (volumetric flow rate (mL/min) × 60 (min/hr))/cross sectional area (cm^2)

*Dynamic binding conditions (10% breakthrough):
Sample: 1mg/mL GST (26kDa) pure protein in 50mM Tris, pH 8.0 and 150mM NaCl
Column dimensions (w × h): 5mm × 50mm (1mL resin)
Flow rate: 0.5mL/min

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce

Important Product Information

- Protein yield and purity are dependent upon the expression level, conformation and solubility characteristics of the recombinant fusion protein, as well as the buffer conditions and flow rates used. 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. Decreasing the flow rate during the sample load will increase binding capacity.
- To avoid sample loss, try to not exceed the maximum resin-binding capacity for the target protein for the purification conditions used. Volumes will vary based on the protein and expression efficiency and will have to be determined and optimized for each over-expressed protein. Typically over-expressed proteins represent 1-30% of the final sample protein concentration. Adjust resin volume as appropriate; total available capacity should be 20-40% higher than what is needed.
- Optimization of cell lysis procedures 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 sonication or French press. Confirm cell disruption before proceeding to protein purification. Add EDTA-free protease inhibitors, such as Thermo Scientific Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (Product No. 78437), during lysis procedures to protect proteins from degradation.
- For liquid chromatography (LC) applications, use highly pure buffer components and water. Degas or filter buffers through a 0.45µm filter before use.

Recommended Buffers

Native conditions:

- Equilibration and Wash Buffer: 125mM Tris-HCl, 150mM sodium chloride; pH 8.0
- Elution Buffer: 125mM Tris-HCl, 150mM sodium chloride, 10mM reduced glutathione; pH 8.0

Regeneration:

- Regeneration Buffer: 125mM Tris-HCl, 150mM sodium chloride; pH 8.0
- Ultrapure water

Clean-in-place:

- 6M guanidine•HCl
- 70% ethanol in ultrapure water
- Ultrapure water

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

Note: Monitor and collect all fractions during a purification to avoid accidental loss of target protein. User can adjust sample collection based on their needs and comfort level with the purification methods used. Maximum flow rates will be dependent on application and equipment used. The procedure may be performed at room temperature or 4°C.

Additional Materials Required

- Suitable LC system
- Empty column for resin packing (follow column manufacturer's protocol for packing)
- Recommended buffers (see above) and volumes (see below)

1. Pack an appropriate-sized column with resin according to column manufacturer's protocol. Ensure the packing flow rate is at least 20% faster than the flow rate that will be used during purification.

2. Equilibrate the column and all buffers to working temperature. Purifications can be performed at room temperature or at 4°C. Ensure all solutions are degassed.
3. Prepare the LC system by washing pumps and filling tubing with buffer. To avoid introducing air into the system, allow a few drops of buffer to flow from the tubing into the column top. Connect column to the tubing.
4. Equilibrate the column with 5-10 column volumes of the Equilibration/Wash Buffer at a flow rate of 300cm/hr or less.
5. Apply any sample volume that does not exceed column-binding capacity for target protein at a flow rate of 150cm/hr or less.

   Note: Binding capacity is flow rate- and protein-dependent. Decreasing the flow rate during the sample load will increase binding capacity. Higher flow rates will decrease production time but will result in a small portion of the target protein in the flow-through fraction.

   Note: For maximum binding, prepare sample by mixing protein extract 1:1 with Equilibration/Wash Buffer (to adjust the sample to the ionic strength and pH of the Equilibration/Wash Buffer) before applying to the column.

   Note: If the sample contains insoluble matter, centrifuge or filter (0.45µm filter) before use.
6. Wash the resin at a flow rate of 150cm/hr or less with 10-15 column volumes of Equilibration/Wash Buffer or until the absorbance approaches baseline.
7. Elute at a flow rate of 150cm/hr or less with approximately 5-10 column volumes of Elution Buffer and collect fractions.

   Note: Monitor protein elution by measuring the absorbance of the fractions at 280nm. The eluted protein can be directly analyzed by SDS-PAGE. To remove excess glutathione for downstream applications, use gel filtration or dialysis (e.g., Thermo Scientific Zeba Spin Desalting Columns or Slide-A-Lyzer Dialysis Cassettes (see the Related Thermo Scientific Products Section).
8. Regenerate column by washing with 10 column volumes of Regeneration Buffer at a flow rate of 150cm/hr or less. The column is now ready for reuse (proceed to Step 1), storage (proceed to Step 9) or routine clean-in-place procedures (see Procedure for Resin Cleaning-in-Place Section).
9. For storage, equilibrate the column with 5 column volumes of ultrapure water containing 0.05% sodium azide or 20% ethanol. Seal column and store at 4°C.

Procedure for Purification of GST-Tagged Proteins by Batch Method

Note: The procedure may be performed at room temperature or 4°C. The Pierce Glutathione Superflow Agarose allows for customization of your purification strategy. Purification conditions can be scaled as needed in several formats. A batch method based on centrifugation is included below. Alternatively, methods based on vacuum filtration or gravity flow can be used to collect flow-through, wash and elution fractions.

Additional Materials Required

- Suitable vessel or sample-handling containers, such as centrifugation bottle or spin filters/columns
- Recommended buffers (see Recommended Buffers Section) and volumes (see below)
- End-over-end rotary mixer or equivalent mixing apparatus

1. Add the required amount of Glutathione Superflow Agarose to a container with 3-4 times the volume of the resin quantity used. Centrifuge for 2 minutes at 700 × *g* and carefully remove and discard the supernatant.
2. Add two resin-bed volumes of Equilibration/Wash Buffer and mix until the resin is fully suspended.
3. Centrifuge for 2 minutes at 700 × *g* and carefully remove and discard buffer.
4. Prepare sample by mixing the protein extract with Equilibration/Wash Buffer to a volume greater than or equal to the resin-bed volume.
5. Add the prepared protein extract to the tube and mix slowly for 30 minutes to 2 hours ensuring the resin stays suspended. For best results, use an end-over-end rotary mixer.

6. Centrifuge for 2 minutes at 700 × *g* and carefully remove supernatant. If desired, save supernatant for downstream analysis.
7. Wash the resin with two resin-bed volumes of Equilibration/Wash Buffer. Centrifuge for 2 minutes at 700 × *g* and carefully remove supernatant. If desired, save supernatant for downstream analysis.
8. Repeat wash step three times.
9. Elute bound GST-tagged proteins by suspending resin bed in one resin-bed volume of Elution Buffer. Mix slowly for 10 minutes making sure resin stays suspended.
10. Spin tube for 2 minutes at 700 × *g*. Carefully remove and save the supernatant.
11. Repeat elution Steps 9-10 two to four times, saving each supernatant fraction in a separate tube.
12. 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. To remove excess reduced glutathione for other downstream applications, use gel filtration or dialysis (e.g., Zeba™ Spin Desalting Columns or Slide-A-Lyzer™ Dialysis Cassettes; see the Related Thermo Scientific Products Section).
13. Regenerate the resin by suspending resin with 10 resin-bed volumes of Regeneration Buffer. Centrifuge tube for 2 minutes at 700 × *g*. Discard supernatant. Repeat once. The column is now ready for reuse (proceed to Step 1), storage (proceed to Step 14) or routine clean-in-place procedures (see Procedure for Resin Cleaning-in-Place Section).
14. For storage, suspend resin with 5 column volumes of ultrapure water containing 0.05% sodium azide or 20% ethanol. Seal column and store at 4°C.

Procedure for Resin Cleaning-in-Place

The Glutathione Superflow Agarose can be used multiple times without affecting protein yield or purity. To prevent cross contamination of samples, designate a given column to one specific fusion protein. If an increase in backpressure or decrease in performance is observed, the following cleaning procedures can be followed.

1. Wash resin with 2 column volumes of 6M guanidine.
2. Wash resin with 5 column volumes of ultrapure water.
3. Wash resin with 4 column volumes of 70% ethanol.
4. Wash resin with 5 column volumes of ultrapure water.
5. Store resin in ultrapure water containing 0.05% sodium azide or 20% ethanol at 4°C.

Troubleshooting

Problem	Cause	Solution
Low protein yield	Poor expression of soluble protein	Optimize expression conditions (temperature of induction, length of induction, media, cell type)
	Insufficient cell lysis and extraction	Optimize cell lysis protocol
	Fusion protein did not bind to the column	Verify the sequence
		Perform an ELISA or Western blot using an antibody against the GST tag to ensure the GST tag is present
	Flow rate was too fast	Decrease flow rate during binding to allow for greater residence time and increased binding of fusion protein
	Insufficient washing	Increase duration of wash
Poor protein purity	Column was dirty	Follow clean-in-place procedure to remove nonspecifically bound proteins
	Column was overloaded	Apply less protein extract onto the column
Slow column flow	Extract was too viscous or highly particulate	Dilute lysate with Equilibration Buffer to decrease viscosity
		Centrifuge lysate at higher speed to remove particulate
		Use Thermo Scientific Pierce Universal Nuclease for Cell Lysis (Product No. 88700-2) to eliminate DNA/RNA
	Column was dirty after multiple uses	Perform clean-in-place procedure

Related Thermo Scientific Products

16100-2 **Pierce Glutathione Agarose**

16103-5 **Pierce Glutathione Spin Columns**

16106-8 **Pierce GST Spin Purification Kit**

90078-9 **B-PER™ Bacterial Protein Extraction Reagent with Enzymes**

78248 **B-PER Bacterial Protein Extraction Reagent**

87786 **Halt™ Protease Inhibitor Cocktail (100X)**

78259 **Glutathione (reduced)**

30001 **Pierce Anti-Glutathione S-Transferase Antibody**

88946-7 **Pierce HRV 3C Protease**

32520 **Factor Xa (Bovine)**

22660 **Pierce 660nm Protein Assay Reagent**

23238 **Coomassie Plus™ (Bradford) Protein Assay Reagent**

89934 **Zeba Desalting Chromatography Cartridges, 7K MWCO, 1mL**

89893 **Zeba Spin Desalting Columns, 7K MWCO, 10mL**

87717 **Slide-A-Lyzer G2 Dialysis Cassettes, 2K MWCO, 0.5mL**

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