INSTRUCTIONS

Pierce® GST Agarose
(Glutathione S-Transferase)

20205 20211

0693.2

Number	Description
20205	**Pierce Glutathione S-Transferase (GST) Agarose Columns,** 2 × 2mL prepacked columns with twist-off bottom tab and accessory pack containing two white tips
20211	**Pierce Glutathione S-Transferase (GST) Agarose,** 5mL resin
	Support: 6% crosslinked beaded agarose
	Loading: 2mg of GST coupled per milliliter of settled resin
	Capacity: A minimum of 1mg of antibody molecules per 2mL of resin recognizing GST from goat anti-GST serum
	Supplied: 50% aqueous slurry containing 0.1% sodium azide
	Storage: Upon receipt store at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce GST Agarose is glutathione S-transferase from *Schistosoma japonicum* that has been covalently immobilized on crosslinked, 6% beaded agarose. Protein expression in *E. coli* using GST-fusion systems is well established and becoming the method of choice to study protein function, purify interacting factors and generate antibodies against fused proteins.[1] Using a specific endopeptidase site, the GST portion can be cleaved from the fused protein by thrombin or factor Xa. However, more often the entire purified GST-fusion protein is used as an immunogen for antibody production. The antibodies generated against such fusion proteins recognize not only the fused protein but also the GST portion. This can create cross-reactivity problems in Western blot analyses, immunohistochemistry and immunoprecipitation. Thus, antibody molecules recognizing the GST portion must be removed from the sample.[2]

Antibodies recognizing GST can be removed from the sample by affinity purification using immobilized GST. Antibodies against GST will bind to immobilized GST whereas antibodies against fusion proteins will not. If desired, the bound GST antibodies can be recovered using 6M guanidine•HCl. The immobilized GST can be regenerated and reused a minimum of nine times.

Procedure for Affinity Purification Using a 2mL Gravity-flow Column

Note: If using Product No. 20211 to prepare a different size column, adjust volumes of sample and buffers accordingly.

Additional Materials Required

- Binding/Wash Buffer: Thermo Scientific BupH Phosphate Buffered Saline Pack (Product No. 28372) or 0.1M sodium phosphate, 0.15M NaCl, pH 7.2
- Elution Buffer: 6M guanidine•HCl (8M guanidine•HCl is available as Product No. 24115)
- Storage Buffer: 0.05% sodium azide

A. Affinity Purification

Note: Perform chromatography at 4°C.

1. Remove top cap and twist off bottom tab from column. Place column in a test tube and allow storage solution to drain.
2. Equilibrate column with 10mL of Binding/Wash Buffer. Do not allow resin to run dry; always recap column or proceed to the next step as soon as solution drains down to top of resin bed. White tips are supplied as bottom caps.

3. Mix equal volumes of antiserum and Binding/Wash Buffer. For optimal results, mix 3mL antiserum with 3mL Binding/Wash Buffer.
4. Apply buffered sample to the column.
5. Collect 0.5-1.0mL fractions. Monitor fractions by measuring the absorbance at 280nm relative to a buffer blank. All fractions above baseline contain protein without antibodies against GST.
6. Wash the column with 10mL of Binding/Wash Buffer, continuing to collect fractions.
7. Pool fractions that contain protein.

B. Elution of Bound Anti-GST Antibody

1. Apply 10mL of 6M guanidine•HCl to the column. Collect 0.5-1.0 fractions. These fractions will contain undesired antibodies that recognize the fusion protein's GST portion.
2. Measure the absorbance of fractions at 280nm to verify the presence of the undesired anti-GST.

C. Column Regeneration and Storage

Note: To regenerate column, the anti-GST antibody must be eluted as described in Section B.

1. Apply 10mL of water, followed by 10mL of the Binding/Wash Buffer.
2. For storage, wash the column with 10mL of 0.05% sodium azide in water or buffer.
3. Cap column when approximately 1mL of solution remains above the top porous disc.
4. Store column upright at 4°C. Immobilized GST can be regenerated and reused a minimum of nine times.

Related Thermo Scientific Products

16100	**Pierce Glutathione Agarose,** 10mL
16105	**Pierce Glutathione Spin Columns, 3mL,** 5/pkg
30001	**Pierce Anti-Glutathione-S-Transferase Antibody, 0.1mg, mouse IgG1 (clone G172-1128)**
89897	**Pierce Centrifuge Columns, 5mL,** 25/pkg

Additional Information Available on Our Website

- Tech Tip #7: Remove air bubbles from columns to restore flow rate
- Tech Tip #29: Degas buffers for use in affinity and gel filtration columns

Cited References

1. Smith, D.B. and Johnson, K.S. (1988). Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. *Gene*, **67:** 31-40.
2. Bar-Peled, M. and Raikhel, N.V. (1996). A method for isolation and purification of specific antibodies to a protein fused to the GST. *Anal Biochem* **241:** 140-2.