Revised: 24–April–2002

Glutathione Agarose, Linked through Sulfur

G-2879 glutathione agarose, linked through sulfur
G-21800 glutathione agarose, linked through sulfur *bulk packaging*

Quick Facts

Storage upon receipt:
- 4°C
- Do not freeze
- Protect from light

Introduction

A popular method for obtaining large amounts of a desired protein is to express the foreign gene in *Escherichia coli*. Glutathione *S*-transferase (GST) gene fusion systems have been widely used for this purpose. The overexpressed fusion protein, which contains a GST "tail," may be purified from the bacterial lysate in a single step by affinity chromatography on glutathione agarose.[1-7] Molecular Probes' glutathione agarose is especially designed for the purification of GST recombinant proteins and other glutathione-binding proteins (Figure 1). To aid researchers purifying GST-fusion proteins, Molecular Probes also offers a highly purified and high-titer anti-GST polyclonal IgG antibody (A-5800). This antibody can be used for the specific detection of GST-fusion proteins by Western blot before and after purification. We also offer this anti-GST antibody conjugated to our green-fluorescent Alexa Fluor® 488 dye (A-11131) for detection of GST-fusion proteins.

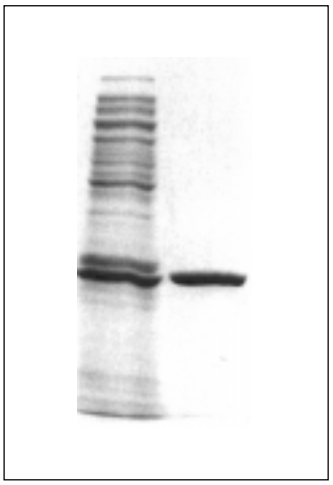

Figure 1. *Coomassie® Brilliant Blue–stained SDS-polyacrylamide gel demonstrating the purification of a GST fusion protein using Molecular Probes' glutathione agarose. Lane 1 contains crude supernatant from an E. coli lysate; lane 2 contains the affinity-purified fusion protein.*

GST-fusion proteins are typically eluted from glutathione agarose with excess glutathione. Alternatively, the GST-fusion expression vector can be engineered to encode a recognition site for a specific protease, such as thrombin or factor Xa, between the GST structural gene and the gene of interest.[8-11] Once the fusion protein is bound to the affinity matrix, the site-specific protease can be added to release the desired protein, leaving the GST bound to the matrix.

Materials

Molecular Probes' glutathione agarose consists of glutathione linked via the sulfur atom to crosslinked beaded agarose. Each mL of gel can bind approximately 5–6 mg bovine liver GST. Adding excess free glutathione liberates the GST fragment from the matrix, which can then be regenerated by washing with a high-salt buffer.

Contents and Storage

Glutathione agarose is provided in either a 10 mL or a 100 mL unit size as a sedimented bead suspension equilibrated in 10 mM potassium phosphate, 150 mM NaCl, pH 7.2 (PBS), containing 2 mM sodium azide to inhibit bacterial growth. The material should be stored at 4°C, protected from light. DO NOT FREEZE. When stored properly, the product should remain stable for at least six months.

Affinity Chromatography

The following protocol briefly describes the methodology for affinity chromatography with glutathione agarose. It is intended as an introductory guide only and may need modification to suit particular experimental conditions and buffer systems.

1.1 After cell lysis, the sample must be centrifuged to remove any undissolved membranes and cellular debris before being applied to the column. Much of the fusion protein may be inadvertently discarded with the pellet in this step.[12,13] If analysis of the pellet indicates the presence of a substantial amount of the desired protein, the fusion protein may often be recovered by resuspending the pellet in a solution comprising 1.5% *N*-lauroylsarcosine, 25 mM triethanolamine and 1 mM EDTA, pH 8.0, mixing for 10 minutes and then recentrifuging at 10,000 × g for 10 minutes at 4°C.[12] Addition of Triton® X-100 to the supernatants may help to keep the fusion protein in solution.

1.2 Wash the column with 5–10 bed volumes of PBS to remove azide.

1.3 Equilibrate the gel bed with 3–5 bed volumes of PBS containing 1% Triton X-100.

1.4 Apply the appropriate amount of sample to the column. The flowthrough (and the wash fraction of step 1.5) may be collected as a control.

1.5 Wash the column with 10 bed volumes of PBS, or until no protein can be detected in the eluate.

1.6 Elute the bound proteins with 5 bed volumes of 50 mM Tris-HCl buffer, pH 8, containing 5 mM glutathione (approximately 1.5 mg/mL glutathione) and collect the fractions.

1.7 If the gel is to be used again, it should be regenerated immediately after step 1.6 by washing the column with PBS containing 3 M NaCl. After thorough washing, the column should be equilibrated in PBS containing 2 mM sodium azide and stored at 4°C. Repeat step 1.2 prior to the next use.

References

1. European Patent No. 0 293 249 B1; **2.** Biochemistry 30, 3674 (1991); **3.** Cell 64, 521 (1991); **4.** J Immunol Methods 136, 211 (1991); **5.** Science 252, 712 (1991); **6.** Gene 67, 31 (1988); **7.** Proc Natl Acad Sci USA 83, 8703 (1986); **8.** J Biol Chem 270, 24525 (1995); **9.** J Cell Biol 129, 189 (1995); **10.** Mol Biol Cell 6, 247 (1995); **11.** Biochemistry 31, 5841 (1992); **12.** Biotechniques 13, 856 (1992); **13.** Biotechnol Bioeng 39, 828 (1992).

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
A-5800	anti-glutathione *S*-transferase, rabbit IgG fraction *3 mg/mL*	0.5 mL
A-11131	anti-glutathione *S*-transferase, rabbit IgG fraction, Alexa Fluor® 488 conjugate *2 mg/mL*	0.5 mL
G-2879	glutathione agarose, linked through sulfur *sedimented bead suspension*	10 mL
G-21800	glutathione agarose, linked through sulfur *sedimented bead suspension* *bulk packaging*	100 mL

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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