Product Information

Glutathione-Agarose

Lyophilized powder

G4510

Product Description

Affinity chromatography with glutathione-agarose permits rapid, mild, non-denaturing and highly selective purification of glutathione-binding enzymes,[1-4] such as glutathione-S-transferase, glutathione peroxidase, and glyoxalase I.

This product consists of glutathione attached through its sulfur to epoxy-activated 4% cross-linked beaded agarose, resulting in a 12-atom (10-carbon) spacer. Glutathione-Agarose is provided as a lyophilized powder stabilized with lactose. Lactose is added as a stabilizer to prevent bead fracture. One gram of powder swells to 10.5-16.0 mL of gel (~70 mg of powder swells to 1 mL of gel).

Several publications,[5-15] theses,[16-21] and dissertations[22-42] have cited use of this G4510 product in their protocols.

Product Profile

- Ligand: 10 to 30 µmoles per mL swollen resin
- Binding capacity: 5-10 mg glutathione S-transferase per mL resin

Storage/Stability

If stored properly desiccated at –20 °C, the product has a shelf life of two years. If exposed to heat and moisture, the beads may not swell properly, or may not be active.

After the resin has been swollen, it can be stored in 2 M NaCl at 2-8 °C. For long-term storage, a bacteriostatic agent, such as 1 mM sodium azide, should be included. Alternatively, the resin can be re-lyophilized after the addition of lactose.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Glutathione-Agarose gel

- Swell the lyophilized powder in water at 200 mL/g. (Use of some buffers can result in non-recoverable loss of volume.)
- Typically, 90-95% of swelling occurs within 30 minutes at room temperature. However, it may require overnight at 2-8 °C for 100% swelling to occur.
- After swelling, the agarose beads must be washed thoroughly with 10 volumes of water or Equilibration Buffer (such as PBS), to remove the lactose present in the lyophilized product.

Buffers and Other Reagents

- Equilibration Buffer: Phosphate buffered saline (PBS, such as Cat. No. P3813), 10 mM phosphate buffer, pH 7.4, 150 mM NaCl. Add appropriate protease inhibitors when preparing cell lysate.
- TRITON™ X-100: Cat. No. T9284
- PBS containing 1% TRITON™ X-100
- Elution Buffer: 5-10 mM reduced glutathione (Cat. No. G4251) in 50 mM Tris-HCl (pH 9.5 ± 0.1 at 4 °C, or pH 9.0 at 25 °C).
- Cleansing Buffer 1: 0.1 M borate buffer, pH 8.5, containing 0.5 M NaCl. Prepare using boric acid (Cat. No. B0394). Adjust pH with NaOH.
- Cleansing Buffer 2: 0.1 M acetate buffer, pH 4.5, containing 0.5 M NaCl. Prepare acetate buffer using sodium acetate (such as Cat. No. 236500). Adjust pH with acetic acid (such as Cat. No. 695092).
- Storage Buffer: 2 M NaCl containing 1 mM sodium azide as preservative

Procedure

Glutathione-agarose may be used in column or batch purifications. This procedure may be used as a guideline for column purification. For batch purifications, use low speed centrifugation (10 seconds at 200-500 × *g*) to pellet the beads, and decant between steps.

Column Set-Up

1. Prepare resin as described in the Preparation Instructions.
2. De-aerate the resin. Carefully pour the slurry into the column, avoiding the introduction of air bubbles.
3. After the resin has been packed in the column, equilibrate the resin with several column volumes of Equilibration Buffer. Do not let the column run dry at any time.

Purification of GST

1. Prepare cell lysate in appropriate buffer.
   - Tris or phosphate buffers, pH 6.5-9.5, are typical lysis buffers compatible with glutathione affinity chromatography.
   - Salt concentrations of up to 1 M do not interfere with binding.
   - Protease inhibitors such as EDTA (such as Cat. No. E7889) or PMSF (Cat. No. P7626) are often included in the lysis buffer. Serine protease inhibitors included in the lysis buffer will not interfere with subsequent thrombin or factor Xa treatment, as these inhibitors are removed before the proteolysis step.
   - The binding of GST to glutathione-agarose is unaffected by 1% TRITON™ X-100, 1% CTAB, 1% TWEEN®-20, 10 mM DTT, or 0.03% SDS.
2. Add TRITON™ X-100 to a final concentration of 1% (v/v).
3. Centrifuge 10 minutes at 10,000 × *g* at 4 °C, or filter through a 0.45 µm filter to clear cell lysate.

   Note: Use only clarified supernatant. To prevent clogging the column, highly viscous samples containing chromosomal DNA or RNA should be sonicated or treated with nuclease to reduce the viscosity. Cellular debris and particulate matter must be removed by centrifugation or filtration.
4. Load the clarified supernatant onto the column under gravity flow.

   Note: Depending on the sample and the flow rate, not all the protein may bind. Multiple passes over the column or closing the loaded column and incubating it on a rotator, may improve the binding efficiency.

   For batch purification, add 2 mL of glutathione-agarose (50% v/v) in Equilibration Buffer to an appropriate amount of clarified supernatant. Incubate sample with glutathione-agarose with gentle mixing for 5-30 minutes at 4 °C.
5. Wash resin four times with PBS-T at 4 °C.
6. Elute GST from the resin with Elution Buffer (3 times, 1 mL each). For batch purification, mix each elution step gently for 2 minutes.
7. Analyze each fraction by SDS-PAGE.
8. Free glutathione may be removed from sample by dialysis against buffer of choice.

Column Cleaning

1. Wash with ~5 resin volumes of Cleansing Buffer 1.
2. Wash with at least 5 column volumes of water.
3. Wash with at least 5 column volumes of Cleansing Buffer 2.
4. Wash with ~5 resin volumes of water. For long-term storage, store in 2 M NaCl containing a bacteriostatic agent.
5. Equilibrate with Equilibration Buffer before use.

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