Product Information

Glutathione-Agarose

Set of 3 pre-packed columns (2.5 mL each),
1:1 suspension in a 0.5 M NaCl + 20% ethanol solution

G3907

Product Description

Affinity chromatography with glutathione-agarose permits rapid, mild, non-denaturing and highly selective purification of glutathione-binding enzymes,[1-12] such as glutathione-S-transferase, glutathione peroxidase, and glyoxalase I. This product consists of pre-packed columns where the resin has glutathione attached through its sulfur to epoxy-activated 4% cross-linked beaded agarose, to give a 12-atom spacer.

Several publications have cited use of this G3907 product in their protocols.[13-14]

Product Profile

- Three columns pre-packed with 2.5 mL each of a saline suspension of glutathione-agarose
- Ligand: 10 to 20 µmoles per mL swollen resin
- Binding capacity: ~5-10 mg glutathione S-transferase per mL resin

Storage/Stability

If stored properly at 2-8 °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.

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

Reagents

Equilibration Buffer: Phosphate buffered saline (PBS), 10 mM phosphate buffer (pH 7.4) and 150 mM NaCl (such as Cat. No. P3813). Add appropriate protease inhibitors when preparing cell lysate.

Triton X-100: Cat. No. T9284.

PBS-T: PBS containing 1% Triton™ X-100.

Elution Buffer: 5 mM to 10 mM reduced glutathione (such as Cat. No. G4251) in 50 mM Tris-HCl, pH 8.0, prepared fresh. Final pH: ~7.5.

Cleansing Buffer 1: 0.1 M borate buffer, pH 8-9, with 0.5 M NaCl. Prepare using boric acid (such as Cat. No. B0394). Adjust pH with NaOH.

Cleansing Buffer 2: 0.1 M acetate buffer, pH 4, with 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: 1 M NaCl with 1 mM sodium azide as preservative.

Procedure

1. Equilibrate the resin with several column volumes of Equilibration Buffer. Do not let the column run dry at any time.
2. Prepare cell lysate in appropriate buffer.
   - Tris or phosphate buffers, pH 6.5-9.5, are typical lysis buffers which are compatible with glutathione affinity chromatography.
   - Salt concentrations of up to 1 M do not interfere with binding.
   - Protease inhibitors such as EDTA (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.
   - Binding of GST to glutathione-agarose is unaffected by 1% Triton™ X-100, 1% Tween®-20, 1% CTAB, 10 mM DTT, or 0.03% SDS.
3. Add Triton™ X-100 to a final concentration of 1% (v/v).

4. 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.

5. 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.

6. Wash resin four times with PBS-T at 4 °C.
7. Elute GST from the resin with Elution Buffer (3 times, 1 mL each). For batch purification, mix each elution step gently for 2 minutes.
8. Analyze each fraction by SDS-PAGE.
9. Free glutathione may be removed from sample by dialysis against buffer of choice.

Regeneration

1. Wash with approximately 5 resin volumes of 0.1 M borate buffer, pH 8-9, containing 0.5 M NaCl (Cleansing Buffer 1).
2. Wash with at least 5 column volumes of deionized water.
3. Wash with at least 5 column volumes of 0.1 M acetate buffer, pH 4, containing 0.5 M NaCl (Cleansing Buffer 2).
4. Wash with approximately 5 resin volumes of deionized water. For long-term storage, store in 1 M NaCl containing a bacteriostatic agent.
5. Equilibrate with Equilibration Buffer before use.

References

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