Glutathione Beads

Cat. 1005-5/10/25

GST-tag affinity purification gel

Version 1.3 03.07

Introduction

Adar Biotech's **Glutathione Beads** are used for the purification of glutathione S-transferase (GST) tagged proteins, produced using various commercial expression vectors. GST-fused proteins can be purified from bacterial lysates by one-step affinity purification.

Adar biotech **Glutathione Beads** is state of the art affinity-resin product that consists of glutathione attached through sulfur to an epoxy-activated 4% cross-linked beaded agarose. The use of the **Glutathione Beads** enables the purification of various GST-fused proteins, while maintaining mild, non-denaturing conditions throughout the purification process.

Glutathione Beads characteristics.

Matrix: Sepharose CL-4B
Activation method: Oxiran.
Binding capacity: 4-8 mg/ml recombinant GST.
Bead size: 40-165 μm
Bead structure: Highly cross-linked spherical agarose, 4%
Max back pressure: 0.3 MPa, 3 bar
Max. flow rates: 4 ml/min/cm^2
Recommended flow rate: 1-2 ml/min/cm^2
Stability of the matrix: pH 4-10.
Storage: 4°C in PBS pH 7.4 added with NaN_3 0.1% (w/v) as preservatives.

Protocol: GST-tagged protein purification

A. Buffers needed

Lysis buffer (5 ml): Your choice of lysis buffer (preferably does not contain reducing agents and chelators).

Column equilibration buffer (100 ml): Phosphate buffer saline (PBS) pH 7.4
Binding buffer (10 ml): PBS pH 7.4
Wash buffer: (200 ml): PBS pH 7.4 optional PBS plus 1% Triton X-100.
Elution buffer (200 ml): 10 mM reduced Glutathione in 50mM Tris-HCl, pH 8

Regeneration buffer (200 ml): 0.1 M borate buffer pH 4.5 plus 0.5 M NaCl.
Storage buffer: 2 M NaCl plus 0.1% w/v sodium azide as preservative

B. Cell lysis (recommended protocol)

1. Thaw frozen cells pellet and resuspend in 5 ml of Lysis buffer. Bacterial culture volume processed for purification may be between 50-250 ml in volume. Thaw cell pellet and suspend in lysis buffer of your preference (not supplied). Lysis buffers may contain reducing agents or chelators. Mix thoroughly to create homogenate.
2. Sonicate or homogenize on ice to lyse cells (i.e. six 10 sec long sonication cycles with 5 sec pauses between).
3. Centrifuge lysate at 10,000 x *g* for 20 min at 4°C. Collect supernatant. Save 20 µl of the supernatant for SDS-PAGE analysis. Place collected supernatant on ice, or freeze until purification column is ready to use.

C. Purification

1. Mix 5 ml of the Glutathione Beads slurry thoroughly until homogeneous suspension is visible. Transfer the gel suspension into an appropriate column with inner diameter of 1.0 to 1.5 cm.
2. After column preparation equilibrate the column with Binding buffer by washing with 5-10 column volumes. Recommended flow rates are 1-2 ml/min/cm^2.
3. Bring the sample to room temperature. Apply the sample to column at a rate between 0.2 ml/min to 0.5 ml/min, using a syringe or a pump. The total volume of the sample applied is not critical in most cases. Save the flow through fraction for SDS-PAGE analysis.
4. Wash with x20 column volumes of Wash buffer (not supplied).
5. Elute with Elution buffer (not supplied), at flow rates of 1-2 ml/min/cm^2. Two to five column volumes are usually needed for elution of the GST- tagged protein. Use the elution buffer as blank when doing the quantization of the target protein in eluted fractions.
6. Removal of Glutathione from eluted fraction. Use your method of choice (buffer exchange by gel filtration or dialysis) to remove glutathione from eluted fraction.

D. Re-equilibration and Storage

1. Strip the column by washing it with 10 column volumes of Regeneration buffer.
2. Wash the column with 10 volumes of Column Equlibration buffer.
3. Storage conditions: Store column in a refrigerator with Storage buffer.