Datasheet for ABIN1536559

Glutathione Resin

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Overview

Quantity:	10 mL
Application:	Pull-Down Assay (Pull-Down)

Product Details

Specificity:	Glutathione Resin is an affinity chromatography medium designed for easy, one-step purification of recombinant glutathione S-transferase (GST) fusion proteins and other glutathione binding proteins expressed in E. coli, insect cells and mammalian cells. The recombinant GST fusion proteins can be purified directly from pre-treated cell lysate using Glutathione Resin. It is the excellent choice for high performance purifications.
Characteristics:	Resin volume: 10 ml settled resin (20 ml 50% slurry) Ligand: Glutathione Dynamic binding capacity: > 20 mg horse liver GST (26 kDa)/ml settled resin Matrix: 4% cross-linked agarose Average particle size: 90 µm Key features: Easy to use: Simple and fast procedures for purification of GST-fusion proteins High capacity: The system can support over 20 mg horse liver GST/ml medium Stability: This reusable resin shows no obvious decrease of the binding capacity after three uses
Bead Ligand:	Glutathione
Bead Matrix:	Agarose beads
Bead Size:	90 µm

Application Details

Reagent Preparation:	Regeneration and Storage of Glutathione Resin Glutathione Resin can be reused to purify the same protein three times without regeneration. If the target GST-fusion protein is different, however, the Glutathione Resin must be regenerated using the following protocol: 1. Wash the column with 2×bed volumes of 0.1 M Tris HCl + 0.5 M NaCl, pH 8.5. 2. Wash the column with 2×bed volumes of 0.1 M sodium acetate + 0.5 M NaCl, pH 4.5. 3. Re-equilibrate the column with 3-5×bed volumes of 1×PBS. 4. For long-term storage, the resin should be stored in 1×PBS containing 20% ethanol at 2 - 8°C.
Sample Preparation:	Preparation of Cell Extract 1.Harvest cells by centrifugation at 3,000 g at 4°C for 10 min, remove and discard the supernatant. 2. Resuspend the cell pellet in 3 ml ice-cold 1×PBS buffer per 50 ml culture and centrifuge at 3,000 g at 4°C for 10 min. Remove and discard the supernatant. 3. Freeze the cell pellet at -80°C for 1 hour (This is also a convenient point to stop and one can continue the procedure later). 4. Thaw cell pellet on ice and resuspend cells in 3 ml of ice-cold 1×PBS buffer per 50 ml culture. If desired, add appropriate additives, such as non-ionic detergents (NP-40) or protease inhibitors (PMSF). 5. Disrupt cells by brief pulses of sonication on ice until the sample is no longer viscous. 6. Centrifuge at 12,000 g at 4°C for 10 min and carefully transfer the supernatant (soluble fraction) to a clean and pre-chilled tube and resuspend pellet (insoluble fraction) with 3 ml of ice-cold 1×PBS buffer per 50 ml culture. 7. Aliquot 10 µl samples from both soluble and insoluble fractions for SDS-PAGE analysis [by adding equal volume of 2X SDS Sample Buffer (125 mM Tris-HCl, pH 6.8, 4% w/v SDS, 20% glycerol, 100 mM DTT,0.02% w/v bromophenol blue), boiling for 5 min and running SDS-PAGE to determine the amount and solubility of the GST-fusion protein]. Note: 1. The binding of GST or GST-fusion protein to Glutathione Resin is not affected by 1% Triton X-100, 1% Tween-20, 1% CTAB, 10 mM DTT, 0.03% SDS, or 0.1% NP-40. These chemicals may be used to reduce non-specific binding. 2. If the target GST-fusion protein forms inclusion body (insoluble protein), the inclusion body has to be properly solubilized and refolded prior to purification.
Assay Procedure:	Purification of Recombinant GST-Fusion Protein 1. Completely resuspend the Glutathione Resin by gently shaking the vial.

Application Details

	2. Transfer an appropriate amount of slurry to a disposable column (included in Kit L00207 and L00208). Usually 1 ml settled resin (2 ml 50% slurry) can bind more than 6 mg horse liver GST protein. 3. Wash the Glutathione Resin with 10×bed volumes of cold (4°C) 1×PBS. 4. Apply clear solution (sonicate, etc) containing GST-fusion protein in cold 1×PBS to the equilibrated column with the flow rate at 10-15 cm/h. 5. Add 1×PBS to wash the column just after all the protein solution get into the column, use 20×bed volumes of PBS for wash. Protease inhibitors such as PMSF are better added to wash solution to inhibit protease activity. 6. Elute the fusion protein with 10-15×bed volumes of freshly made 10 mM glutathione elution buffer (0.154 g of reduced glutathione dissolved in 50 ml of 50 mM Tris-HCl, pH 8.0.). 7. Monitor elution of the fusion protein using absorbance readings at 280 nm. 8. Aliquot 10-20 µl supernatant containing GST-fusion protein, flow-through, wash and the eluted protein, respectively, and analyze all the samples by running SDS-PAGE to confirm the presence of the target protein. 9. Pool eluted fractions containing target protein. Remove free glutathione by dialysis at 4°C against a buffer of choice or by using a G15 Sephadex desalt column.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	1X PBS containing 20% ethanol
Storage:	2-8 °C
Storage Comment:	For long-term storage, the resin should be stored in 1×PBS containing 20% ethanol at 2 - 8°C.
Expiry Date:	18 months

Publications

Product cited in:	Zhang, Zou, Ma, Muhammad, Li, Jiang: "Identification of Mycoplasma suis MSG1 interaction proteins on porcine erythrocytes." in: **Archives of microbiology**, Vol. 197, Issue 2, pp. 277-83, (2015) (PubMed). Theoret, Uzal, McClane: "Identification and characterization of Clostridium perfringens beta toxin variants with differing trypsin sensitivity and in vitro cytotoxicity activity." in: **Infection and**

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Spagnol, Reiling, Kieken, Caplan, Sorgen: "Chemical shift assignments of the C-terminal Eps15 homology domain-3 EH domain." in: **Biomolecular NMR assignments**, Vol. 8, Issue 2, pp. 263-7, (2014) (PubMed).

Smardon, Diab, Tarsio, Diakov, Nasab, West, Kane: "The RAVE complex is an isoform-specific V-ATPase assembly factor in yeast." in: **Molecular biology of the cell**, Vol. 25, Issue 3, pp. 356-67, (2014) (PubMed).

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