INSTRUCTIONS

Glutathione Magnetic Beads

Catalog Number: AE05001 AE05002

AvanBio Inc.
239 New Road, Suite A-107
Parsippany, NJ 07054
U.S.A.

Phone: +1 (800) 278-9865
Fax: +1 (855) 378-6845
Email: support@avanbio.com

【Introduction】

EmerTher Glutathione Magnetic Beads are nano-superparamagnetic beads coupled with reduced glutathione. The product provides an efficient and reliable method to purify GST (glutathione-*S*-transferase)-tagged fusion proteins from crude cell lysates. The beads are simply added to the crude cell lysate and the GST-tagged fusion proteins bind to the beads. After washing the unbound proteins off, the target GST-fusion protein is recovered by elution with 50mM glutathione.

【Product Specifications】

Diameter：500nm
30 min sedimentation rate：<0.1%
Magnetic response rate: >30emu/g
pH stability：pH 3-13
Storage solvent： 20% ethanol
Binding capacity: 10-100μg GST-tagged fusion protein per mg magnetic beads

【Product Content】

Catalogue Number	Conc. (mg/ml)	Volume (ml)	Amount of Beads (mg)
AE05001	50	1	50
AE05002	50	4	200

【Purification Protocol】

The following protocol provides general guidelines for purification of GST-tagged fusion proteins using EmerTher Glutathione Magnetic Beads. For a specific fusion protein, please adjust bead quantities and other conditions based on the expression level and the nature of the target protein. The protocol is scalable.

A. Note:

1. Elution Buffer contains glutathione (see below) should be prepared freshly.
2. EmerTher Glutathione Magnetic Beads can be re-used after regeneration of the beads.
3. Boiling the beads in SDS-PAGE reducing sample buffer will cause loss of binding activity of the beads, in which case the beads can only be used for one time. .
4. If the target GST-fusion protein is solubilized in urea or guanidine, these denaturants need to be removed through dialysis. The protein needs to refold to resume the function of GST prior to the affinity purification using the beads.

B. Additional materials recommended:

1. Binding/Washing Buffer: 0.15M NaCl，125mM Tris-HCl，pH 8.0
2. Elution Buffer: 50mM glutathione，0.25M NaCl，10mM Tris-HCl，use 1N KOH adjust pH to 8.5
3. Storage Buffer after regeneration of the beads: 20% ethanol
4. A magnetic stand

C. Purification procedure:

1. Gently mix the magnetic beads thoroughly before use by repeated inversion or using a rotating device.
2. Place 20μl of magnetic beads (1mg) into a 1.5ml microcentrifuge tube.
3. Place the tube on a magnetic stand, collect the beads and discard the supernatant.
4. Wash the beads three times with Binding/Washing Buffer (500μl each time) by magnetic separation, and re-suspend the beads in 200-750μl of Binding/Washing Buffer.
5. Add the same volume (200-750μl) of cell lysate to the bead suspension; mix thoroughly and incubate for 30min at room temperature on a rotator.
6. Collect the beads with a magnet and save the supernatant for analysis if desired.

7. Wash the protein-coupled beads three times with Binding/Washing Buffer (1ml each time), incubate for 5 min each time before applying magnet to separate beads from buffer.
8. Suspend the beads in 100µl of Elution Buffer, incubate for 15 min. Apply magnet and transfer the supernatant to a clean microcentrifuge tube. Repeat this step once if desired. Combine the eluates from multiple elutions.
9. The purified protein is ready for use (e.g. determination of protein concentration or evaluation by SDS-PAGE).

D. Regeneration of the glutathione magnetic beads:

1. After elution of the protein, add 500µl urea (6M) to the beads and incubate for 10min.
2. Collect the beads with a magnet and re-suspend the beads in 500µl NaCl solution (0.5M, containing 0.1% SDS), incubate for 5 min, then collect the beads with a magnet. Repeat this step once.
3. Wash the beads three times with Binding/Washing Buffer (500µl each time) by magnetic separation. Re-suspend the beads in Storage Buffer. Store at 4º C until ready for use.

【Storage】

Stored at 2-8 °C, 2 years

【Troubleshooting】

A. Low recovery of GST-fusion protein after the purification using the beads
   1. Protein may degrade. Add protease inhibitors to cell lysate preparations.
   2. Protein is expressed at a high level. Increase the amount of magnetic beads accordingly.
   3. Insufficient target GST-fusion protein is present in sample. Increase the amount of sample.

B. GST-fusion protein did not elute from the beads
   1. Large protein generally elute less efficiently. Increase the concentration of glutathione in Elution Buffer to 100mM.
   2. Elution condition is too mild. Add NaCl (up to 250mM) to increase the ionic strength of Elution Buffer; add 0.1-1% Triton X-100 or Tween-20 detergent in Elution Buffer; increase incubation time with Elution Buffer; or increase the volume of the Elution Buffer.
   3. Proteins are more efficiently elute at pH ≥8.0. Increase the pH of the Elution Buffer may increase recovery.

C. Multiple non-specific proteins are observed in eluted sample
   1. Protein may degrade. Add protease inhibitors to cell lysate preparations.
   2. Nonspecific proteins bind to the beads. Add NaCl (up to 350mM) to the Binding/Washing Buffer to increase stringency.

Find out more at AvanBio

This product is for research use only, and is not for use in diagnostic procedures. Please visit our website at www.avanbio.com for MSDS information and more life science research products from AvanBio. For technical support, please email us: support@avanbio.com.