MCLAB
Molecular Cloning Laboratories

User Manual

Version 3.0

Product name: Glutathione Agarose Beads

Cat #: GAB-100, GAB-200, GAB-300, GAB-OEM

Description:

Glutathione S-transferase (GST) gene fusion systems have been widely used for obtaining large amounts of desirable protein in expressed *Escherichia coli*. The fusion proteins, which contain a GST tail can then be purified through affinity chromatography. MCLAB's glutathione agarose is designed for the specific purification of GST recombinant proteins and other glutathione-binding proteins. Glutathione agarose is uniquely formulated for excellent binding capacity and purity of the proteins of interest.

Protocol:

The following instructions for GST-fusion proteins purification can be scaled up or down depending on the users preference. This manual exemplifies a sample preparation from a specific amount of starting material and purification using 1 ml resin.

1. Centrifuge the sample after cell lysis to remove undissolved membranes and cellular debris before applying to the purification column.
2. Wash the purification column with 10x bead volumes of Binding Buffer to remove azide.
3. Dilute an appropriate amount of the sample with a 1:1 ratio of Binding Buffer before applying to the purification column.
4. Wash the purification column with 10x bead volumes of Binding Buffer or until no proteins can be detected in the washes.
5. Elute the bound protein of interest with 5x bead volumes of Elution Buffer.
6. GST agarose beads can be saved for later use by washing the purification column with Binding Buffer containing 3 M NaCl. After a thorough wash, the purification column should be equilibrated in Binding Buffer containing 2 mM sodium azide and stored at 4°C.
   GST-fusion proteins are generally eluted from glutathione agarose beads with the use of excess glutathione. Alternatively, GST-fusion protein can be encoded with a cleavage site between the GST and the protein, allowing the desirable protein to be eluted with the use of a protease.

Recommended Storage Condition: 4 °C.

Binding Buffer:

50 mM Tris
150 mM NaCl
pH 8.0

Elution Buffer:

50 mM Tris pH 7.8
0.15 M NaCl
1 mM EDTA
1 mM DTT
10 mM GST