Glutathione Affinity Superflow 4

Glutathione Affinity Superflow 4 is a resin used for the purification of a variety of different Glutathione (GST) tagged proteins including: Glutathione S-transferases, Glutathione-dependent proteins, and recombinant derivates of Glutathione S-transferases.

Glutathione Affinity Superflow 4 purification requires some degree of method development for optimization. The protocol below is meant as an example for binding GST tagged proteins.

Protocol:

Reagents:

Wash buffer:	PBS (1X)
Binding buffer	PBS (150 mM NaCl, 3 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4), pH 7.35.
Elution buffer:	50mM Tris-HCl, 10mM reduced glutathione, pH = 8.0.

***** If necessary, the addition of dithiothreitol (DTT) to either the wash and/or elution buffer can reduce the risk of oxidation of free thiol (-SH) groups on GST. This dimerization of the GST-tagged proteins can result in precipitation and lower yields. It is recommended that 1-20 mM DTT be used. *****

Purification of GST Tagged Proteins

1. Pour the desired amount of Glutathione Affinity Superflow 4 in a Buchner funnel.
2. Wash resin with 10 bed volumes (BV) of wash buffer to remove all residual 20% ethanol.
3. Suction dry resin and remove to a designated container.
4. Add the cell lysate to the resin.
5. Rock/mix for 30-45 minutes at room temperature to ensure complete equilibration.
6. Remove sample and suction dry resin. Make sure to collect flow-through for further analysis.
7. To the resin, add 3 BV of wash buffer and mix for 3-5 minutes at room temperature.
8. Repeat steps 6-7 three additional times and collect fractions. This will result in the removal of any unbound material.
9. Elute the bound protein by adding elution buffer in a 1:1 ratio to Glutathione Affinity Superflow 4 resin.
10. Rock/mix for 30 minutes at room temperature to ensure complete equilibration.
11. Remove sample and suction dry resin. Make sure to collect flow-through for further analysis.
12. Repeat steps 9-11 three additional times and collect fractions.
13. Analyze each fraction for purified protein by measuring absorbance at A_{280}.
14. Collect and pool fractions of purified protein together.

Method for Cleaning/Regeneration:

1. Wash the resin with 3-5 BV of a solution containing: 0.1M Tris-HCl 50mM Tris, 0.5M NaCl, pH 8.5.
2. Wash the resin with 3-5 BV of DI water.
3. Wash the resin with 3-5 BV of 0.1M Sodium Acetate, 1M NaCl, pH 4.5.
4. Wash the resin with 3-5 BV of DI water.
5. Wash the resin with 3-5 BV of wash buffer to re-equilibrate.

For technical service email info@sterogene.com or call (760) 929-0455