Glutathione-Magnetic Nanoparticles

Description

1 ml product per vial, supplied at 10 mg/ml in storage buffer (5 mM Sodium phosphate buffer, pH 8 containing 20% Ethanol)

Storage: Upon receipt store at 4°C.

Important Product Information

- Do not centrifuge, dry or freeze the product.
- XpressAffinity Glutathione-Magnetic Nanoparticles are compatible with small-scale purification of GST-tagged fusion proteins.
- To minimize protein degradation, include protease inhibitors in the preparation of cell lysate.
- Boiling the nanoparticles in SDS-PAGE reducing sample buffer is acceptable for single-use applications as it results in nanoparticle aggregation and loss of binding capacity.

GST-Tagged Fusion Protein Purification from Cell Lysate

Additional Materials Required

- 1.5 ml micro-centrifuge tubes
- Cell lysate
- Binding Buffer: Buffer used to prepare cell lysate
- Wash Buffer: 1X PBS or 5mM Sodium phosphate buffer, pH 8
- Elution Buffer: 20 mM Glutathione, 50 mM Tris, 300 mM NaCl, 1 mM EDTA, 1% Triton X-100 pH 8 (Freshly prepared)
- Magnetic stand (MagnaStand; Cat No. MG17MS12)
- Tube rotator

Protocol

Note: *Mix the nanoparticles thoroughly before use by repeated inversion or gentle pipetting* ***(do not vortex)****.*

1. Prepare the cell lysate containing the GST-tagged fusion protein (**following your lab protocol**).
2. Place 100 µl of XpressAffinity Glutathione-Magnetic Nanoparticles into a sterile 1.5 ml micro-centrifuge tube (for purifying approximately 0.5 mg of recombinant protein).

Note: *Optimal amount of nanoparticles required for each purification should be standardized by the user based on the amount of GST -tagged fusion protein in the cell lysate.*

3. Place the tube on a magnetic stand and allow the nanoparticles to stick to the side of the tube. Discard the clear supernatant.
4. **Wash:** Add 500 µl of Binding buffer to the nanoparticles and gently invert the tube to mix. Place the tube on a magnetic stand and allow the nanoparticles to stick to the side of the tube. Discard the clear supernatant.
5. Repeat wash (Step 4) twice.
6. **Binding:** Add the prepared cell lysate to the tube containing the nanoparticles, gently mix to re-suspend completely (**do not vortex**) and incubate at 4^0C with rotational mixing from 2 hours to overnight (using a tube rotator at 10-20 rpm speed).
7. After incubation, place the tube on the magnetic stand and allow the nanoparticles to stick to the side of the tube. Save the supernatant for further analysis, if required.
8. **Wash:** Add 500 µl of Wash buffer to the tube and gently invert the tube to mix. Place the tube on the magnetic stand and allow the nanoparticles to stick to the side of the tube. Discard the clear supernatant.
9. Repeat wash (Step 8) twice.
10. **Elution:** Add 400 µl of Elution buffer to the tube and incubate 1 hour at room temperature with rotational mixing (using a tube rotator at 10-20 rpm speed).

Note: *Volume of the Elution buffer should be standardized by the user based on the expected concentration of the purified protein.*

11. Place the tube on the magnetic stand and allow the nanoparticles to stick to the side of the tube. Collect the supernatant containing the eluted recombinant protein. Repeat elution, if required.
12. To remove free glutathione and other components from the purified recombinant protein solution, perform a dialysis against a suitable buffer at 4^0C.