Protein A, Protein G or Protein A/G-Magnetic Nanoparticles

Description

1 ml product per vial, supplied at 10 mg/ml in storage buffer (5 mM Sodium phosphate buffer pH 8 containing 0.05% Tween-20 and 0.05% Sodium azide)

Storage: Upon receipt store at 4°C.

Important Product Information

- Do not centrifuge, dry or freeze the product.
- XpressAffinity Protein-Magnetic Nanoparticles are compatible with small-scale antibody purification and immunoprecipitation.
- 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.

Antibody Purification from Serum, Cell Culture Supernatant or Ascites

Additional Materials Required

- 2 ml micro-centrifuge tubes
- Sample: serum, cell culture supernatant or ascites
- Binding/Wash buffer: 5 mM Sodium phophate buffer, pH 8.0
- Elution buffer: preferably use 0.2 M L-Arginine, pH 2.8
- Neutralization buffer: 1 M Tris, pH 9.0
- 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. Place 100 µl of XpressAffinity Protein A, Protein G or Protein A/G-Magnetic Nanoparticles into a sterile 2 ml micro-centrifuge tube.
2. Place the tube on a magnetic stand and allow the nanoparticles to stick to the side of

the tube. Discard the storage buffer of the nanoparticles.

3. **Wash:** Add 1 ml of Binding/Wash buffer and invert/tap the tube several times 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.
4. Repeat wash (step 3) twice.
5. Re-suspend the nanoparticles in 100 µl of Binding/Wash buffer.
6. Add 100 µl of sample to the tube and invert to mix. Make up to 1 ml with Binding/Wash buffer.

Note:

-100-600 µl of sample can be used as required by the experiment.

-For purification of antibodies from serum, we recommend diluting the sample to a final concentration of 1 mg/ml. (For example, 100 µl serum is diluted to a final volume of 1 ml).

-For purification of secreted antibodies in cell culture, we recommend using 1 ml of Protein-Magnetic Nanoparticles for 100-200 ml cell culture supernatant. For purifying antibodies from cell lysate, use 1 ml nanoparticles for 20-25 ml cell lysate.

7. **Binding:** Incubate the sample at room temperature with rotational mixing for 1 hour (using a tube rotator at 10-20 rpm speed).

***Note**: Incubate overnight at 4^0C for larger cell culture supernatant or lysate volumes.*

8. After incubation, place the tube on the magnetic stand and allow the nanoparticles to stick to the side of the tube. Discard the clear supernatant.
9. **Wash:** Add 1 ml of Binding/Wash buffer to the tube, and invert/tap the tube several times 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.
10. Repeat wash (step 9) twice.
11. **Elution:** Add 300 µl of Elution buffer to the tube, mix well and incubate 5 minutes at room temperature with intermittent gentle mixing.
12. 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 antibody (E1). To neutralize the low pH, add the required amount of Neutralization buffer to the eluted antibody.
13. Repeat the elution step and collect the eluted antibody as E2, E3 etc until the IgG gets completely eluted. Neutralize all eluates and proceed with protein

quantification/SDS-PAGE.

Immunoprecipitation

Additional Materials Required

- 1.5 ml micro-centrifuge tubes
- Sample: Cell lysate
- Antibody for immunoprecipitation
- Binding/Wash buffer: Buffer used to prepare cell lysate-antibody mixture or 1X PBS
- 1X SDS-PAGE reducing sample buffer
- Magnetic stand (MagnaStand; Cat No. MG17MS12)
- Tube rotator

Protocol

Note: *This protocol is only a guideline for immunoprecipitation and will require optimization for different types of analyses.*

1. **Incubation of lysate with antibody:** Prepare the cell lysate and incubate with primary antibody against the target antigen (**following your lab protocol**). Incubate at 4^{0}C from a few hours to overnight.
2. Place 20 µl of XpressAffinity Protein A, Protein G or Protein A/G-Magnetic Nanoparticles into a sterile 1.5 ml micro-centrifuge tube.
3. **Wash:** Add 500 µl of Binding/Wash 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.
4. Repeat wash (Step 3) twice.
5. **Binding:** Add the lysate-antibody mixture to the tube containing the nanoparticles, gently mix to re-suspend completely (**do not vortex**) and incubate at 4^{0}C with rotational mixing from 2 hours to overnight (using a tube rotator at 10-20 rpm speed).
6. After incubation, place the tube on the magnetic stand and allow the nanoparticles to stick to the side of the tube. Save the flow-through for SDS-PAGE, if required.
7. **Wash:** Add 500 µl of 1X PBS 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 supernatant completely.

8. Repeat wash (Step 7) twice.
9. **Elution:** Add 50 µl of 1X SDS-PAGE reducing sample buffer to the nanoparticles and heat samples at 96-100ºC in a heat block for 10 minutes.
10. Place the tube on the magnetic stand and allow the nanoparticles to stick to the side of the tube; collect the supernatant containing the target antigen. Proceed with SDS-PAGE and Western blotting.