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NBP1-71715 Protocol

Immunoprecipitation (NBP1-71715)

Note: This protocol is a general guideline for immunoprecipitation and will require optimization for each application.

1. Combine the antigen sample with 10ug of antibody. Adjust the reaction volume to 500uL with the Cell Lysis Buffer. Incubate the reaction for 1-2 hours at room temperature or overnight at 4C with mixing.

2. Place 25uL (0.25mg) of Protein A/G Magnetic Beads into a 1.5mL microcentrifuge tube.

3. Add 175uL of Wash Buffer (Tris-buffered saline containing 0.05% Tween-20 Detergent and 0.5M NaCl) to the beads and gently vortex to mix.

4. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant

5. Add 1mL of Wash Buffer to the tube. Invert the tube several times or gently vortex to mix for 1 minute. Collect beads with magnetic stand. Remove and discard the supernatant.

6. Add the antigen sample/antibody mixture to a 1.5mL microcentrifuge tube containing pre-washed magnetic beads and incubate at room temperature for 1 hour with mixing.

7. Collect the beads with a magnetic stand and then remove the flow-through and save for analysis.

8. Add 500uL of Wash Buffer to the tube and gently mix. Collect the beads and discard the supernatant. Repeat wash twice.

9. Add 500uL of purified water to the tube and gently mix. Collect the beads on a magnetic stand and discard the supernatant.

10. Low-pH Elution: Add 200uL of Elution Buffer (1% SDS, 100mM NaHCO3) to the tube. Incubate the tube at room temperature with mixing for 15 minutes. Magnetically separate the beads and save the supernatant containing target antigen.