Immunoprecipitation using Protein A/G Magnetic Beads

Overview

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Introduction

Use 25 µl of Protein A or Protein G Magnetic Beads per 200 µl of crude cell lysate containing 200-500 µg of total protein in a standard immunoprecipitation protocol. It is important to increase the volume of beads proportionately for larger cell lysate volumes.

Protocol

1. Cell Lysis

   (1) Rinse a 60 mm culture dish of confluent cells with PBS.
   (2) Lyse the cells with 0.5 ml cold Immunoprecipitation Buffer (150 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM EGTA (pH 8.0), 0.2 mM sodium ortho-vanadate, 0.2 mM PMSF, 1% Triton X-100, 0.5% NP-40).
   (3) Maintain constant agitation for 30 minutes at 4°C.
   (4) Scrape the cells from the dish. Sonicate on ice for 5 seconds; repeat 4 times. Centrifuge for 5 minutes at 4°C. The supernatant is the crude cell lysate. Assay for total protein then adjust concentration to approximately 1 mg/ml with Immunoprecipitation Buffer.

2. Immunoprecipitation

   (1) This step pre-clears crude cell extract of proteins which can bind non-specifically to the beads. In a 1.5 ml microcentrifuge tube, add 25 µl Protein A/G Magnetic Beads to 200µl of crude cell extract. Gently vortex and incubate at 4°C for 1 hour. Apply magnetic field for 30 seconds to pull beads to the side of the tube. Pipette supernatant to a clean 1.5 ml microcentrifuge tube. Discard beads.
   (2) Add 1-5 µg of antibody to crude cell lysate vortex and incubate at 4°C for 1 hour. (If monoclonal antibodies are used, add 5 µg rabbit anti-mouse IgG antibody. Vortex and incubate an additional 30 minutes at 4°C).*
   *Alternatively, Protein G Magnetic Beads (NEB #S1430S) can be used for immunoprecipitations with monoclonal antibodies.
   (3) Add 25 µl of Protein A/G Magnetic Beads suspension. Gently vortex and incubate with agitation for 1 hour at 4°C.
   (4) Apply magnetic field to pull beads to the side of the tube. Carefully pipette to remove supernatant.
   (5) Wash with 500 µl of Immunoprecipitation Buffer by gentle vortex. Apply magnetic field then remove supernatant and discard. Repeat wash 2 times.
   (6) Resuspend bead pellet in 30 µl of 3X SDS Sample Loading Buffer (187.5 mM Tris-HCl (pH 6.8), 6% (w/v) SDS, 30% glycerol, 150 mM DTT, 0.03% (w/v) bromophenol blue, 2% β-mercaptoethanol).
   (7) Incubate sample at 70°C for 5 minutes.
   (8) Apply magnetic field to sample then load supernatant on SDS-PAGE gel and electrophorese.