Immunoprecipitation assays(about S100A6 in SW480 Cells)

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Abstract

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Protocol

Cell Lysis

Step 1.

1.Rinse a 60 mm culture dish of confluent cells with PBS

2.Lyse the cells with 0.5 ml cold Immunoprecipitation Buffer containing 1% SDS, 1 mmol/L of Na3VO4, 0.1 mol/L of Tris (pH 7.4), and protease inhibitors (10 mg/L of leupeptin, 5 mg/L of aprotinin, 20 mg/L of soybean trypsin inhibitor, and 1 mmol/L of phenylmethylsulfonyl fluoride).

3.Maintain constant agitation for 30 minutes at 4°C.

4.Scrape the cells from the dish.

5.Sonicate on ice for 5 seconds; repeat 4 times

6.Centrifuge for 5 minutes at 4°C

7.Assay for total protein then adjust concentration to approximately 1 mg/ml with Immunoprecipitation Buffer

Immunoprecipitation

Step 2.

8.In a 1.5 ml microcentrifuge tube, add 20 µL of protein A+G Agarose Beads and transferred to a fresh 1.5 mL tube.

9 centrifuging at 1500 rpm for 30 sec at 4 °C and washed three times with 500 µL of lysis buffer.

10. Carefully pipette to remove supernatant

11. Add 2 µg of antibody or 2 mg of IgG to crude cell lysate

11. Incubate overnight at 4 °C.

12. Wash with 500 µl of Immunoprecipitation Buffer by gentle vortex and remove supernatant and discard repeated three times

13.Resuspend bead pellet in 20 µl of 1X SDS Sample Loading Buffer

14.Incubate sample at 70°C for 5 minutes.

15.analysis on SDS-PAGE gel and electrophorese.