Immunoprecipitation assays (about S100A6 in SW480 Cells)

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Works for me

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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

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.