

Immunoprecipitation Protocol

Buffers and Reagents:

Wash buffer: PBS <http://www.fitzgerald-fii.com/pbs-10x-concentrate-85r-125.html>

Cell Lysis Buffer: 0.1% SDS, 0.5% Deoxycholic Acid Sodium Salt, 1% NP-40, 150 mM NaCl, and 50 mM Tris-HCl

Protease Inhibitors

Primary antibody: See our selection of monoclonals at <http://www.fitzgerald-fii.com/monoclonal-antibodies.html> or polyclonals at <http://www.fitzgerald-fii.com/polyclonal-antibodies.html>

Some other useful reagents: <http://www.fitzgerald-fii.com/diluents.html>

General Procedure:

1. Prepare cell lysate with lysis buffer containing protease inhibitor.
2. Preclear 100-500 µg of cell lysate with 100 µl of Protein G Sepharose at 4°C for 1 hr under rotary agitation. The amount of cell lysate will be chosen depending on the abundance of protein.
3. Incubate 1-5 µg of antibody with 10 µl of Protein G Sepharose at 4°C for 1 hr under rotary agitation. The amount of antibody will be chosen depend on the affinity of the antibody for the protein.
4. Immunoprecipitate precleared cell lysate with antibody conjugated Protein G Sepharose at 4°C over night under rotary agitation.
5. Wash Protein G Sepharose with lysis buffer.
6. Add 2X sample buffer and boil at 95°C for 5 minutes.
7. Subject to SDS-PAGE.
8. If predicted molecular size is similar to heavy or light chains of IgG, we recommend to use the secondary antibody that does not recognize denatured heavy and light chains of IgG for western blotting.