

Western Blot Protocol

Buffers and Reagents:

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

Membrane: PVDF Nitrocellulose Membrane

Blocking solution: 5% Skimmed Milk/PBS-T

Wash solution: PBS-T (or TBS-T) PBS or Tris -buffered saline (pH 7.4) with 0.05% (v/v) Tween 20

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>

Secondary antibody: See our full list of conjugated secondary antibodies at <http://www.fitzgerald-fii.com/secondary-antibodies.html>, and choose by source animal, conjugated substance for your choice.

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

General Procedure:

Sample Preparation

1. Wash the sub-confluent cells with ice-cold PBS.
2. Lyse cells with buffer containing 1% SDS and protease inhibitor.
3. Homogenize and sonicate the cell lysate.
4. Perform a Bradford or Lowry assay to determine the protein concentration levels.
5. Add a loading buffer containing SDS and Beta-ME into the whole cell lysate.
6. Boil the mixture for 5 minutes at 95°C.

Electrophoresis

1. Electrophorese according to standard protocols.
2. Transfer proteins from the gel to a PVDF membrane using an electroblotting apparatus.
3. Incubate the membrane in 5% skim milk/TBS-T for 30 minutes at room temperature.

Immunoblotting

1. Incubate the blocked membrane in primary antibody diluted in 1% skim milk/TBS-T for 1 hour at room temperature. Try a range of dilutions (eg. 0.2-2 (µg/ml) and optimize the dilution according to the results.
2. Wash membrane three times for 5 minutes each time with TBST.

3. Incubate the membrane for 30 minutes at room temperature with HRP conjugated secondary antibody diluted in 1% skim milk/TBST.
4. Wash membrane four times for 5 minutes each time with TBST.
5. Incubate membrane in chemiluminescence reagent and visualize proteins using image analyzer.