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BIO MATRIX RESEARCH ™

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Western blot

Sample preparation

1. Wash the sub-confluent cells with ice-cold PBS.
2. Lysed cells with buffer containing 1% SDS and protease inhibitor.
3. Homogenized and sonicated well the cell lysate.
4. To determine the protein concentration levels, performed a Bradford assay or a Lowry assay.
5. Added a loading buffer containing SDS and β-ME into the whole cell lysate.
6. Boiled the mixture for 5 minutes at 95℃.

Electrophoresis

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

Immunoblotting

1. Incubated the blocked membrane in primary antibody diluted in 1% skim milk/TBST for 1 hour at room temperature. Try a range of dilutions (0.2-2 μg/ml) and optimize the dilution according to the results.
2. Wash membrane three times for 5 minutes each with TBST.
3. Incubation 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 with TBST.
5. Incubated membrane in chemiluminescence reagent and visualized proteins using image analyzer.