Western Blot Analysis

In 2 collections

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

Western blot protocol

1 Wash cells 1X in PBS.

2 Detach cells using Accutase for 5 minutes at 37°C and collect them.

3 Spin cells in a centrifuge at 250g for 5 minutes at room temperature.

4 Remove the supernatant.

5 Lyse cells in 1% TBS + 0.5% NP40 + PI/PHI (Pierce #A32959).

6 Determine protein concentration was determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific).

7 Dilute samples in 6x Laemmli buffer containing 12.5% β-mercaptoethanol.

8 Boil samples 5 minutes at 95°C in a thermoblocker.

9 Load protein in a self-casted 7.5-15% acrylamide gel or in precast NuPage 4-12% Bis-Tris Protein gel.

10 Run gel at 100V for 30min-2h.

11 Transfer proteins to PDVF membrane via wet transfer (20% methanol) overnight at 20V at 4°C.

12 Block membrane in TBS 0.1% Tween containing 5% milk or 5% bovine serum albumin.

13 Incubate primary or secondary antibodies in 5% Roche Block solution or 5% BSA in TBS-T supplemented with 0.04% sodium azide.