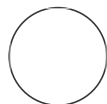




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Western Blot Analysis

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ABSTRACT

Western blot protocol

OPEN  ACCESS**DOI:**dx.doi.org/10.17504/protocols.io.j8nlkwp515r/v1**Protocol Citation:** michela.deleidi, Federico Bertoli 2023. Western Blot Analysis.**protocols.io**<https://dx.doi.org/10.17504/protocols.io.j8nlkwp515r/v1>**License:** This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited**Protocol status:** Working

We use this protocol and it's working

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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.