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A General Protocol for Western Blotting Mammalian Cell Lysates

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David Dilworth[1]

[1]Structural Genomics Consortium

David Dilworth

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

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Harvesting and Lysis

1 For adherent cell lines, wash with 1 x PBS and detach cells by incubating in 0.25% trypsin. Once cells have detached at an equal volume of complete media and transfer to a microfuge tube. Suspension cells can be transferred directly to microfuge tube from culture. Spin cells at 500 x G for 5 min to pellet. Wash pellet with 1 x PBS and repeat centrifugation followed by removal of supernatant.

2 Resuspend cell pellet in 50 µL - 100 µL lysis buffer. Incubate on ice for 10 minutes and then add SDS to 1% final.

Note

Lysis Buffer:
* 20 mM Tris-HCl pH8
* 150 mM NaCl
* 10 mM MgCl2
* 1mM EDTA
* 0.5 % Triton X-100
Add fresh protease Inhibitors (100x) & benzoase (10 000x) prior to lysis.

3 Quantify and normalize protein concentration between samples using the bicinchoninic acid assay (BCA assay) - Pierce BCA Protein Assay Kit (Cat# 23225)

Western Blotting

4 To each sample add SDS Loading buffer to 1 x and boil samples for 5 min.
98 °C

5 Load SDS page with 50 - 100 µg total protein and run in an appropriate buffer at 100 V for ~ 2 hours, until the dye front runs off. For NuPAGE 4-12% Bis-Tris Protein Gel (NP0322BOX), we run in 1x MOPS Running Buffer.

6 Transfer proteins using appropriate transfer apparatus, for 1.5 hrs at 80 volts in 1 x Tris-Glycine transfer buffer to a 0.2 µm PVDF membrane. For PVDF membranes, pre-soak in 100% methanol followed by transfer buffer.

7 Block membrane in 5% milk in PBS-T (1x PBS - 0.1% Tween-20) for 30 min at RT.

8 Cut membrane and probe with desired antibodies diluted in 5% BSA in PBS-T overnight at 4oC.

9 Wash membranes 3 x - 10min in PBS-T.

10 Incubate membranes in secondary LiCor antibodies to mouse and/or rabbit (diluted - 1:5000) in Licor Odyssey Blocking buffer (927-40000) diluted 1 in 5 in PBS-T.

11 Wash membranes 3 x - 10min in PBS-T.

12 Image blots on Licor Odyssey CLx Imaging System.