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Western Blot - Frozen cell pellets

Cell Genomics

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We use this protocol and it's working

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Abstract

This protocol describes how to extract protein from frozen pellets and how to perform a western blot for protein detection.

Protein Extraction

1h

1 Thaw the cell pellets On ice . 10m

2 Add RIPA buffer supplemented with protease inhibitors, vortex well and lyse the cells on ice for 00:30:00 . 30m

(For a 200,000 cells pellet use 40 µL of lysis buffer, scale up or down accordingly)

3 Centrifuge at 12.000 rpm, 4°C for 00:20:00 20m

4 Carefully transfer the supernatant to a new tube. Store the protein lysate at -80 °C or continue with the steps in the Western Blot section.

Western Blot

6h 25m

5 **Prepare gel samples:** 5m

- Mix the appropriate amount of sample with 4x loading dye (LDS), 10x reducing agent and water.
- Boil the samples for 00:05:00 at 95 °C .
- Spin briefly.

6 **Run the gel:** 45m

- Load the boiled samples in a pre-made 4-12% Tris-glycine SDS-PAGE gel.
- Load a well with a prestained ladder.
- Run at 200V in 1x MES SDS running buffet for 00:45:00 at Room temperature .

7 **Transfer to membrane:** 10m

- Transfer the proteins to a PVDF membrane using the Transblot-Turbo Transfer system (BioRad) following manufacturer's instructions.
- Check the ladder has transferred well to the membrane.

8 **Blocking:** 2h

- Cut the membrane if the size of the proteins to be detected is different enough.

- Place the membrane in a cuvette making sure the protein side is facing up.
- Briefly rinse the membrane with Tris-buffer saline with 0,1% Tween (TBST).
- Block with 5% skimmed milk-TBST for 01:00:00 at Room temperature or Overnight at 4 °C .

9 **Antibody incubation:** 3h 25m

- Dilute primary antibody in 5% skimmed milk-TBST.
- Incubate on a shaker Overnight at 4 °C . For abundant targets, incubate for 01:00:00 at Room temperature .
- Briefly rinse the membrane with TBST.
- Wash three times with TBST on a shaker for 00:10:00 at Room temperature .
- Dilute species-appropriate HRP-conjugated secondary antibody in 5% skimmed milk-TBST.
- Incubate on a shaker for 01:00:00 at Room temperature .
- From this point, protect the membrane from light.
- Briefly rinse the membrane with TBST.
- Wash three times with TBST on a shaker for 00:10:00 at Room temperature .
- Wash once with TBS on a shaker for 00:05:00 at Room temperature .
- NOTE: If using an HRP-conjugated primary antibody, wash thrice and image (skip the secondary antibody step).

10 **Detection:**

- Prepare the detection reagent following manufacturer's instructions.
- Incubate on a shaker for following manufacturer's instructions.
- Image with chemiluminescence using an appropriate imager.

11 **Stripping:**

- Use a commercial stripping buffer as per instruction.
- Briefly rinse the membrane with TBST.
- Repeat blocking, antibody incubation and detection of the new target (usually the loading control).

NOTE: A large proportion of the protein will be lost during the stripping step. Always blot the most abundant target last.