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Western Blot (semi-dry)

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for semi-dry Western Blot

Materials

Semi-dry cathode buffer

- 100 ml 10x conc. Cathode buffer
- 200 ml Methanol
- ad 1L H₂O

Semi-dry anode buffer

- 100 ml 10x anode buffer
- 200 ml Methanol
- ad 1L H₂O

TBST

- 100 mM Tris/HCl pH=8, 150 mM NaCl, 0,1% Tween

TBST + 5% milk powder

Primary / secondary antibodies

Western Blot

- 1 Equilibrate gel in Cathode buffer for 30 minutes (no shaking)
- 2 Activate PCDF-Membrane for ca. 20 seconds in MeOH, incubate for 2 minutes in dH₂O (no shaking)
- 3 Equilibrate PCDF-Membrane for 5 minutes in Anode buffer
- 4 Wet two Blotting papers (thickness 1 mm each) in Anode buffer and place on Anode
- 5 Put PCDF-membrane on top
- 6 Put Gel on top
- 7 Wet two blotting papers (thickness 1 mm each) in Cathode buffer and place on gel.
- 8 Cut membranes and papers as exactly as possible to gel size (Careful! Gel gains some size while equilibrating) and place each component exactly on top of each other. After each step consider eliminating air bubbles by rolling them out with a glass rod with buffer.
- 9 Run blot at 1,5 mA/cm² for 90 minutes (depending on the size of the protein, the blotting duration will have to be adjusted)

Immunodetection

- 10 Block membrane with TBST + 5% milk powder for 45 minutes
- 11 Wash membrane with TBST and primary antibody for at least 1h at RT or 4°C over night. Anti-gfp antibody will be diluted 1:5000 in TBST, use as little volume as possible to preserve the expensive antibody.



- 12 Wash for 10 minutes in TBST. Repeat 3 times.
- 13 Add secondary antibody and incubate at least 45 minutes at RT or at 4°C over night. Swivel gently.
- 14 Wash for 10 minutes in TBST. Repeat 3 times.
- 15 Mix Bio-Rad Clarity Western 1:1 (ca. 1 ml per membrane). Let membrane drain, evenly add developer to membrane (with 1ml tip). Spread between film (remove bubbles), incubate for 1 minutes and detect.
- 16 Blots; Chemi or Chemi-high Sensitivity, Signal Accumulation mode, transmission light for ladder detection
- 17 Don't forget to take a picture of membrane for merge!

Stripping

- 18 Wash blot in TBS
- 19 immerse in stripping buffer
- 20 incubate at RT for 5-15 minutes (Under the hood!)
- 21 remove and wash again in TBS
- 22 reprobe!