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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

- 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

Equilibrate gel in Cathode buffer for 30 minutes (no shaking)
PCDF-Membrane
Activate for ca. 20 seconds in MeOH, incubate for 2 minutes in dH_2O (no shaking)
Equilibrate for 5 minutes in Anode buffer
Wet two Blotting papers (thickness 1 mm each) in Anode buffer and place on Anode
Put PCDF-membrane on top
Put Gel on top
Wet two blotting papers (thickness 1 mm each) in Cathode buffer and place on gel.
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 eachother. After each step consider eliminating air bubbles by rolling them out with a glass rod with buffer.
Run blot at 1,5 mA/cm^2 for 90 minutes (depending on the size of the protein, the blotting duration will have to be adjusted)

Immunodetection

Block membrane with TBST + 5% milk powder for 45 minutes
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.
Wash for 10 minutes in TBST. Repeat 3 times.
Add secondary antibody and incubate at least 45 minutes at RT or at 4°C over night. Swivel gently.
Wash for 10 minutes in TBST. Repeat 3 times.

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.
Blots; Chemi or Chemi-high Sensitivity, Signal Accumulation mode, transmission light for ladder detection
Don't forget to take a picture of membrane for merge!

Stripping

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