

Western blotting

Making gels

1. Wash plates and wipe with ethanol.
2. Place seal between plates and secure with green magnetic clips.
3. Make up running (8%) and stacking gels - leave out **APS** and **TEMED** from stacking gel.
4. Pour running gel. Add some water-saturated butanol to get rid of bubbles. Leave to set for ~20 minutes.
5. Rinse butanol from the gel with water and dry with tissue.
6. Add APS and TEMED to stacking gel. Pour stacking gel with combs in place. Remove any bubbles by knocking plates
7. Loading samples and running gel
8. Put 25 μ l of TCA extract into an eppendorf, with hole in lid. Boil for 3 mins.
9. Remove seal and combs from gels and rinse edges with water. Fix into tank with indented plate facing inwards.
10. Fill tank with 1x Running buffer, filling the centre right to the top.
11. Load 25 μ l to each lane. Also load 25 μ l of markers in marker lanes. Markers are kept in the -20°C freezer.
12. **Remember to number gels**, if running more than one.
13. Run gel: **200V**
 50mA
 ~1hr 30 mins (until blue dye runs off bottom)

100mA.
for x4 gels.

Semi-dry blotting

For each gel:

- 6x 11x9cm blotting paper
- 1x 10x8cm nitrocellulose membrane

Plus:

- Plastic tray containing ethanol
- Plastic tray containing H₂O
- Plastic tray containing transfer buffer

- 1) Soak membrane in ethanol for 30 seconds.
- 2) Rinse in H₂O.
- 3) Soak 3 papers in transfer buffer ~30 seconds.
- 4) Lay down 3 papers on blotting apparatus.
- 5) Soak membrane in transfer buffer.
- 6) Place membrane on top of papers.
- 7) Rinse gel plates, open up and cut off stacking gel.
- 8) Wet gloves to pick up gel and soak it in transfer buffer.
- 9) Place gel on top of membrane. **Label membrane and mark top corner.**
- 10) Soak 3 more papers in transfer buffer and put on top of gel.
- 11) Use a falcon tube to roll out bubbles.
- 12) Put lid onto blotter and screw shut.
- 13) Running conditions: **16V**
250mA
1 hour

PAP 1:500 4 μ l in 2ml marvel
— leave out steps 9 + 10

Blocking and antibodies

- Ponceau stain
- 1x PBS
- Marvel: 25g Marvel (5%)
50ml 10x PBS (1x)
Water to 500mls
- Anti-GFP antibody no.56:
Use **1:500 dilution for cdc18** (4 μ l in 2ml marvel for each membrane),
otherwise **1:2000**
- Secondary antibody: anti-mouse 1:10,000 (0.5 μ l in 5ml marvel).

myc (1:200 10 μ l / 2ml marvel)
1:500 from aug04.
1:10,000 for nda3 block exps

1. To check protein has transferred pour on some Ponceau stain (this is reusable) to visualise bands.
2. Pour off and rinse with 1x PBS.
3. Can take picture on fluorchem of protein bands.
4. 2x washes in 1x PBS on shaker.
5. Block in marvel for 2 hours. Store remaining marvel in fridge.
6. Wash 5 mins in PBS.
7. Place membrane inside plastic cover, wipe out bubbles using tissue. Seal sides of bag. Pour in primary antibody solution. Get rid of bubbles and seal top.
8. Tape to rotating plate, leave at 4°C overnight.
9. 3x 5 min washes in 1x PBS.
10. Rebag membrane with secondary ab. Rotate at room temp 3 hours.
11. 3x 10 min washes in marvel.
12. 3x 5 min washes in 1x PBS
13. Put membrane into a bag with super signal (1ml white bottle and 1ml brown bottle). Remove bubbles and seal. Leave for 5 mins.
14. Pour off excess and put into new bag.
15. Develop film in F-level darkroom (1945).

PICO

WEST DURA SUPERSIGNAL
0.5 ml from each bottle per blot

anti-usignatin 1°
1:1000
2 μ l / 2mls.

anti-Rabbit 2°

Tubulin

1. 20min wash in PBS
2. 3x 10min marvel washes
- ✕ 3. Incubate with anti-tubulin antibody 1:10,000 (^{0.5} ~~1~~ μ l in 5ml). 1 hour room temp.
- ↓ 4. 3x 5min marvel washes.
5. Anti-mouse secondary antibody, 1:10,000 dilution. 1 hour
6. 1x 10 min marvel wash. 2x PBS washes.

* Start here after glycine strip + reBlock 2hr in marvel-