Western Blot (wet transfer)

- solution: transfer buffer: 12.1 g Tris base
  57.67 g glycin
  800 ml MetOH
  add H₂O to 4 l

- use Hoefer transfer chamber (Transphor Electrophoresis Unit TE22)
- prepare nitrocellulose-filter (7x9 cm) and 3 Whatman papers (8x10 cm)
- mark (upper left) corner of nitrocellulose-filter by cutting the corner
- prepare transfer sandwich as follows:

  - built sandwich in a flat container filled with transfer buffer to avoid bubbles
  - fit transfer sandwich in transfer unit and fill with buffer
  - add magnetic stir bar and make sure it can move
  - blot for 30-40 min at 0.5 A (mix buffer and cool with water)
(hint: proteins go to the (+) pole)
- remove blot and open sandwich so that gel lies on top of membrane
- mark the position of the gel on nitrocellulose filter
- gel can be stained with coomassie to check if transfer was complete
- nitrocellulose filters can be used for immunodetection