Western Blot (wet transfer)

- <u>solution:</u> - transfer buffer:

12.1 g Tris base 57.67 g glycin 800 ml MetOH add H_2O 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