

## Wet tank transfer protocol

### 1. Mix up transfer buffer:

#### For 1 gel

50 mL NuPAGE Transfer Buffer  
100 mL Methanol  
850 mL MilliQ Water  
1 mL antioxidant

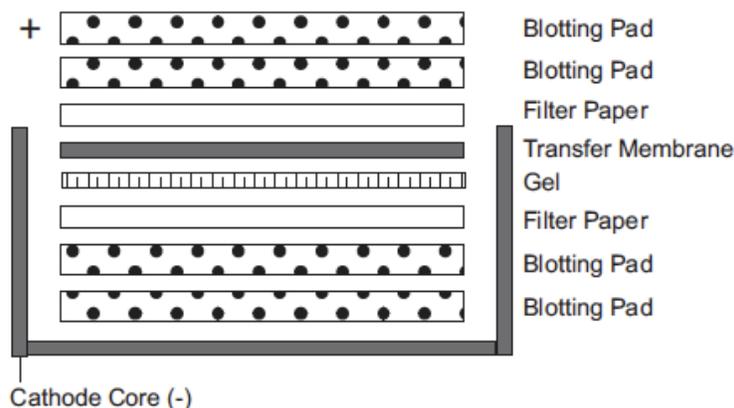
#### For 2 gels

50 mL NuPAGE Transfer Buffer  
200 mL Methanol  
750 mL MilliQ Water  
1 mL antioxidant

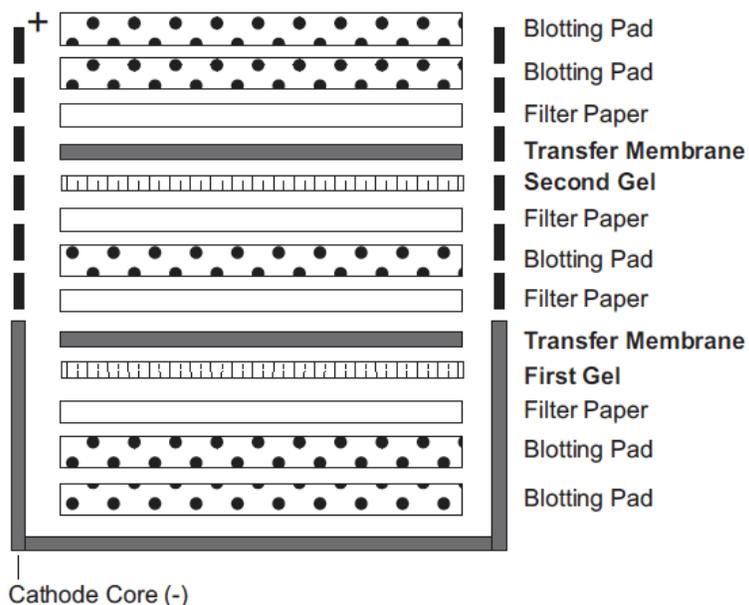
2. Soak nitrocellulose membranes (#LC2000) for 5 min in transfer buffer.
3. Open blot module and place bottom (larger half) face up.
4. Open gel cassette, remove wells and foot (just barely above the foot).
5. Briefly submerge a single piece of filter paper in transfer buffer and place on top of gel.
6. Gently roll filter paper with shortened pipette.
7. Lift up one corner of the gel such that it sticks to the filter paper, peel both away from the bottom of the cassette and flip over. Place back on cassette with the exposed side of the gel facing upwards.
8. Place the soaked membrane on top of the newly exposed gel.
9. Gently roll membrane with shortened pipette. There should be little to no buffer between the membrane and the gel.
10. Briefly submerge and place the second piece of filter paper on top of the membrane.
11. Roll out the completed sandwich as before. The gel/membrane sandwich is now properly oriented for transfer -- DO NOT FLIP!
12. Submerge blot pads in buffer (5 pads needed) and squeeze out all the bubbles (while submerged).
13. Once the bubbles are gone, remove from the buffer and carefully wring out excess buffer.
14. Assemble the stack:

#### For 1 gel

- Place 2 blot pads on the bottom of the blot module (larger side).
- Slide the sandwich on to the top of the blot pads (without flipping).
- Place 2 blot pads on top of the sandwich (3 if needed).
- Close module with other side.



For 2 gels



15. Put the closed module into the gel box and lock it into place.
16. Add transfer buffer into the module until it just covers the blot pads within.
17. Fill the outside of the module with water. This is just a thermal buffer to keep the transfer stack cool.
18. Module is now ready for transfer. Perform transfer in the cold room, or fill the outside of the module with ice before adding water.

Transfer conditions, 1mm gel:

1 gel	30 volts	180 amps	70-75 min
2 gels	30 volts	180 amps	105 min

Transfer conditions, 1.5mm gel:

1 gel	40 volts	190 amps	90-100 min
2 gels	40 volts	190 amps	115 min