

Western Blotting

2/18/09 2:49 PM

Day 1

- Start 5ml overnights

Day 2

- Running the gel
 1. Inoculate 5ml LB
 2. Grow to $OD_{600}=0.3$
 - Record ODs
 3. Pellet 0.5ml
 - To stop here, freeze the pellet at -80°C
 4. 1x Loading Buffer (1ml)
 - 250ul NuPAGE LDS sample buffer 4x (room temp)
 - 100ul 0.5M DTT (-20°C)
 - 650ul dH_2O
 5. Resuspend the pellet in 1x loading buffer
 - Volume of buffer = $OD_{600} \times 500\text{ul}$
 - ex: $0.3 \times 500 = 150\text{ul}$ loading buffer
 6. Sonicate 1 pulse for 5sec at setting 4.5
 7. Heat at 70°C for 10min
 8. Assemble gel chamber
 - Use pre-cast NuPAGE 4-12% Bis-Tris gel
 9. Running buffer
 - 1x MOPS (for large proteins)
 - 1x MES (for $<50\text{kD}$ proteins)
 - Add 500ul NuPAGE antioxidant to 200ml running buffer
 10. Use syringe to wash wells of gel
 11. Load 20ul of each sample (ladder = 0.5ul MagicMark Western Standard + 9.5ul 1x loading buffer)
 12. Run at **200V for 50min**
- Wet transfer
 1. Transfer Buffer
 - 50ml Methanol
 - 25ml NuPAGE 20x transfer buffer
 - 0.5ml NuPAGE antioxidant
 - add dH_2O to 500ml

2. Activate PVDF membrane in EtOH
3. Presoak membrane, filter papers, and 5 sponges in transfer buffer. Push bubbles out of sponges.
4. Open gel case, cut off wells and bottom ridge, and place wet sheet of filter paper on the gel.
5. Peel gel and filter paper off, place wet membrane on gel
6. Place other filter paper on membrane and press/roll out bubbles
7. Place 2 sponges in the transfer apparatus with some transfer buffer
8. Place filter/membrane/gel sandwich on sponges so that the **MEMBRANE IS ON TOP OF THE GEL**
9. Cover with 3 more sponges, close transfer apparatus, rinse gel box with dH₂O, clamp the transfer apparatus in the chamber
10. Fill inner chamber with 1x transfer buffer and outer chamber with dH₂O.
11. Run at **30V for 1hr**

- Blocking and probing

1. 1x Blocking Buffer (50ml)

- 50ml SuperBlock Blocking Buffer in TBS (fridge)
- 250ul Surfact-Amps 20 (10% Tween) (fridge or shelf)

2. 1x Wash Buffer (500ml)

- 1 pouch BupH Tris-Buffered Saline pack
- 500ml dH₂O
- 2.5ml Surfact-Amps 20 (10% Tween)

3. Use 25ml 1x blocking buffer and block for 1hr or overnight on shaker
4. Add 6.8ul anti-VSVG to 15ml blocking buffer. Label for 1hr on shaker. Dispose of primary in hood.
5. Wash 4x on shaker for 10min each
6. Block for 30min on shaker
7. Add 5ul anti-rabbit goat to 50ml blocking buffer (or 1.5ul in 15ml blocking buffer for one blot). Label for 1hr on shaker.
8. Wash 4x on shaker for 10 min each

70ml
75ml
375

9. Mix 5ml of each developer solution and shake on blot for a few minutes before developing.