

Western Blot

1. Sample preparation (4x LDS, DTT, mQ if needed, sample), 70C for 10 min without shaking. Spin 21k rcf for 1 min
2. Remove the comb, remove the tape, place to the tank.
3. Run appropriate time (check on the web site Voltage and time)
4. Prepare the membrane (dump MeOH, take it - reuse it next time, wash mQ 5 min on shaker, dump water, 10 min in transferring buffer, prepare sandwich)
5. Take gel the cassette, break it, cut wells, slot
6. Ice around tank, place in cold room, 70 V, 90 min
7. 5% milk in TBST (w/v) for 1 hour in RT or overnight in a cold room.
8. Fast wash, until tbst will be clean.
9. Primary Ab 1:1000 1 hour RT shaking or overnight 4C (reusable)
10. Wash 3 times 10 min each, change container between 2 and 3 wash
11. Secondary Ab 1:10,000 1h RT shaking (use once),
12. Wash 3 times 10 min each, change container between 2 and 3 wash
13. Add 2-3 ml of HRP substrate evenly across membrane
14. Before visualisation remove HRP substrate by sliding membrane to the walls, and wiping it around with paper
15. Visualisation: adjust the time between the pictures