**Western Blot without *GoBlot***

**Day 1. Duration: ~8 hours**

Gel running and transfer:

 1 Run gels. If you plan to use IR800 detection, use LDS 4x without bromophenol blue.

 2 Transfer proteins from gels to nitrocellulose blots by using *iBlot*

*- For 8 blots, three liters of TBS + 0.1% Tween are needed (this is the “washing buffer”), 85 ml TBS + 0.1% Tween + 5% BSA (this is the “protein block”) and 250 ml TBS + 0.1% Tween + 0.5% BSA (this is the buffer used to dilute the antibodies); BSA:* *Sigma A7906). Also needed: 200 ml of 0.3% H2O2 (in TBS + 0.1% Tween), [20 ml amplification diluent, 10 ml amplification reagent, 50 ml water] and 16 l* *Streptavidin-CW800 conjugate. All steps require rocking @RT.*

Protein blocking, Avidin and Biotin steps:

3 Place each blot in 10 ml of protein block (TBS + 0.1% Tween + 5% BSA [Sigma A7906]). Wait one hour.

4 Wash only once (empty the liquid contents and add 10 ml of washing buffer). Wait 3 min.

5 Empty the liquid contents and add 10 ml TBS + 0.1% Tween plus two drops of Avidin solution (VECTOR). Wait one hour.

6 Wash three times (empty the liquid contents and add 10 ml of washing buffer). Wait 3 min each.

7 Empty the liquid contents and add 10 ml TBS + 0.1% Tween plus two drops of Biotin solution (VECTOR). Wait one hour.

8 Wash three times (empty the liquid contents and add 10 ml of washing buffer). Wait 3 min each.

9 Empty the liquid contents and add 20 ml TBS + 0.1% Tween + 0.3% H2O2. Wait one hour.

10 Wash three times (empty the liquid contents and add 10 ml of washing buffer). Wait 3 min each.

11 Empty the liquid contents and add 10 ml of primary antibody diluted in TBS + 0.1% Tween + BSA 0.5%. Wait overnight.

12 Wash three times (empty the liquid contents and add 10 ml of washing buffer). Wait 3 min each.

13 Empty the liquid contents and add 10 ml of secondary antibody (HRP-conj.) 1/5,000 in TBS + 0.1% Tween + BSA 0.5%. Wait two hours.

14 Wash three times (empty the liquid contents and add 10 ml of washing buffer). Wait 3 min each.

15 Add 10 ml of Bio-Rad Amplification Module (2 parts of 2x “Amplification Diluent”, 1 part of 4x “Amplification reagent” and 5 parts water). Wait one hour.

16 Wash three times (empty the liquid contents and add 10 ml of washing buffer). Wait 3 min each.

17 Add 10 ml Streptavidin-CW800 (1/5,000) in TBS + 0.1% Tween + BSA 0.5%. Wait one hour.

18 Wash three times (empty the liquid contents and add 10 ml of washing buffer). Wait 3 min each.

19 Develop blot by Azure.