**Antibody validation by Western Blot using *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 blots by using *iBlot*

*- For 8 blots, seven liters TBS + 0.1% Tween are needed, 180 ml TBS + 0.1% Tween + 5% BSA and 350 ml TBS + 0.1% Tween + 0.5% BSA (11 gr BSA total). Also, 1,5 liters of 1% SDS (for washing), 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.*

*- Before using the Goblots, perform a Routine #5 using TBS + 0.1% Tween to make sure that all valves are working properly (50 ml in Reservoir Wash Buffer, 10 ml in “Primary antibody” syringe, 10 ml in “Secondary antibody” syringe, and 20 ml in the Reaction Tray). After performing routine #5, empty the Waste Tray.*

Protein blocking, Avidin and Biotin steps (**use Routine #1**):

3 Fill the Reaction Tray of each *GoBlot* with 10 ml of protein block (TBS + 0.1% Tween + 5% BSA [Sigma A7906]). Place blots directly into the Reaction Tray. Add 240 ml TBS + 0.1% Tween in the Reservoir Wash Tray. Make sure that the Waste Tray is empty.

4 Add two drops of Avidin solution (VECTOR) per 10 ml TBS + 0.1% Tween in “Primary antibody” syringe.

5 Add two drops of Biotin solution (VECTOR) per 10 ml TBS + 0.1% Tween in “Secondary antibody” syringe. Press “Start”.

H2O2, primary and secondary antibody (**use Routine #3**), overnight protocol:

6 Empty the Waste Tray. Place new syringes.

7 Add 240 ml TBS + 0.1% Tween in Reservoir Wash Tray.

8 Aspirate contents of Reaction Tray and add 20 ml of 0.3% H2O2 in the Reaction Tray.

9 Add 10 ml of primary antibody diluted in TBS + 0.1% Tween + BSA 0.5% in “Primary antibody” syringe.

10 Add 10 ml of secondary antibody (HRP-conj.) 1/5,000 in TBS + 0.1% Tween + BSA 0.5% in “Secondary antibody” syringe. Cover the Reaction Trays with the lids. Press “Start”.

**Day 2: Duration: ~5 hours**

Amplification and Streptavidin-conjugated HRP (**use Routine #1**):

11 Empty the Waste Tray. Place new syringes.

12 Add 240 ml TBS + 0.1% Tween in Reservoir Wash Tray.

13 Add 10 ml of Bio-Rad Amplification Module (2 parts of 2x “Amplification Diluent”, 1 part of 4x “Amplification reagent” and 5 parts water) in “Primary antibody” syringe.

14 Add 10 ml Streptavidin-CW800 (1/5,000) in TBS + 0.1% Tween + BSA 0.5% in “Secondary antibody” syringe. Press “Start”.

15 Develop blot by Azure.

Washing of GoBlots (**use Routine #5**):

16 Wash all *Goblot*s once by putting 120 ml of 1% SDS in the Reservoir Wash Tray, 10 ml in “Primary antibody” syringe and 20 ml in “Secondary antibody” syringe. Press “Start”.

17 Wash all *Goblot*s with water by putting 120 ml in the Reservoir Wash Tray, 10 ml in “Primary antibody” syringe and 20 ml in “Secondary antibody” syringe. Press “Start”. Repeat step 17 two more times.