Western Blot Procedure

1. Heat diluted samples at 70 degrees Celsius for 10 minutes.

2. Load samples onto a 10% Bis-Tris gel.

3. Run gel for ~45 minutes at 200V.

4. Transfer gel to PVDF membrane at 45V for ~90 minutes.

5. Block the membrane overnight at 4 degrees Celsius, in blocking buffer [PBS + 0.2% Tween-20 + 5% milk], gently shaking.

6. Rinse the membrane twice, quickly, in PBS-T.

7. Wash the membrane in PBS-T 3 times for 5 minutes, each.

8. Incubate the membrane with primary antibody [NB 400-111 or NB 400-118], diluted 1:500 in blocking buffer, for 1 hour at RT, gently shaking.

9. Rinse the membrane twice, quickly, in PBS-T.

10. Wash the membrane in PBS-T 3 times for 5 minutes, each.

11. Incubate the membrane with secondary antibody [NB 730-H], diluted 1:5,000 in blocking buffer, for 1 hour at RT, gently shaking.

12. Rinse the membrane twice, quickly, in PBS-T.

13. Wash the membrane in PBS-T 3 times for 5 minutes, each.