Appendix 2. ELISA working checklist

Day 1

1. Incubate the plate with 100 µL/well of **2 µg/mL EP1802Y Ab**. Seal and store at 4°C overnight.

Day 2

1. Wash plate 3X with 300 µL/well of **wash buffer** and tap dry
2. Block by adding 250 µL/well of **assay buffer** to plate. Seal and incubate at RT for 1-3 hr on benchtop
   Start time: _____
   Sealed: _____ → Stop time: _____
3. Prepare fresh standards from an aliquot of stock rPrP
4. Wash plate 3X with 300 µL/well of **wash buffer** and tap dry
5. Add 100 µL/well of **rPrP standards, mouse QCs, and samples** in duplicate. Seal and incubate at RT for 60-75 min.
   Start time: _____
   Sealed: _____ → Stop time: _____
6. Wash plate 3X with 300 µL/well of **wash buffer** and tap dry
7. Add 100 µL/well of **0.25 µg/mL biotin-8H4 Ab solution**. Seal and incubate at RT for 60-75 mins.
   Start time: _____
   Sealed: _____ → Stop time: _____
8. Wash plate 3X with 300 µL/well of **wash buffer** and tap dry
9. Add 100 µL/well of **24.69ng/mL streptavidin-HRP solution**. Seal and incubate at RT for 30 mins.
   Start time: _____
   Sealed: _____ → Stop time: _____
10. Wash plate 3X with 300 µL/well of **wash buffer** and tap dry.
11. Add 100 µL/well of RT **TMB**. Cover and incubate at RT on benchtop until Std. 1 (5ng/mL) reaches ~0.8 OD (pre-read at 605 nm) or 30 minutes max.
    Start time: _____
    Covered: _____ → Stop time: _____
12. Add 100 µL/well of RT **Stop Solution**. Mix well on plate reader briefly and read at 450 nm and 630 nm.