Appendix 1. Full assay protocol

Abbreviation	Name
Ab	Antibody
BSA	Bovine Serum Albumin
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate
CSF	Cerebrospinal fluid
CV	Coefficient of variation
HRP	Horseradish Peroxidase
LLQ	Lower Limit of Quantification
OD	Optical Density
PBS	Phosphate Buffered Saline
PrP	Prion Protein
QC	Quality Control
RE	Relative Error
rPrP	Recombinant Prion Protein
RT	Ambient Room Temperature
SD	Standard Deviation
TMB	3,3',5,5'-Tetramethylbenzidine
ULQ	Upper Limit of Quantification

Reagents

Name	Manufacturer	Catalog #
Zeba Spin Desalting Columns	Thermo Scientific	89889
EZ-Link Sulfo-NHS-SS-Biotin, No-Weigh format, 1 mg	Thermo Scientific	A39258
1X PBS	Broad Institute	N/A
Pierce BCA Protein Assay Kit	Thermo Scientific	23225
Anti PrP Ab 8H4	Abcam	ab61409

Equipment

Name	Manufacturer	Model #
37°C Incubator	Any	Any
NanoDrop	Thermo Scientific	NanoDrop 8000
SpectraMax M5 Plate Reader	Molecular Devices, Inc.	
Standard Orbital Shaker	VWR	1000

Biotinylation of 8H4 Antibody

Solution Preparations

1. Dilute 90 µg of 8H4 Ab (e.g. 50 µL of 1.8 mg/mL) with 1X PBS to bring to a total 200 µL.

Material Buffer Exchange

2. Remove the bottom closure on the Zeba column and place into a clean 15 mL conical tube. Keep the column upright and cap loosened.

3. Centrifuge the column device at 1000xG for 2 mins. Flow-through is discarded and the device was placed back into the same falcon tube.
4. 1 mL 1X PBS was added directly on top of the resin. The device is centrifuged at 1000 RCF for 2 mins and the flow-through was discarded. This step is repeated two more times for a total of 3 washes.
5. After the last wash step, the column is removed from the conical tube. Keeping the column upright, the bottom of the column is blotted off with a Kimwipe and is transferred to a clean 15 mL falcon tube.
6. 200 µL of 8H4 Ab is applied directly on top of the resin. After 1 min, 40 µL of 1X PBS is applied as a stacker.
7. The device is centrifuged at 1000xG for 2 mins. The column is discarded and the flow-through is kept on ice. The volume collected from the device is measured using a pipette and recorded.

Biotinylation

8. 180 µL of cold Milli-Q water is added into a microtube of 1 mg of NHS-SS-Biotin to prepare a 10mMol Biotin stock solution. The contents are mixed with a pipette and then mini-centrifuged to bring the solution down.
9. **See note for calculations** 14.6 µL of 10mM Biotin stock solution is added into the 8H4 Ab solution and mixed with a pipette.
10. The biotinylated 8H4 Ab solution is covered in foil and placed on the plate shaker for 30 mins at the setting “4” (~127 rpm).

Purification of Conjugated Protein

11. Remove the bottom closure on a new Zeba column and place into a clean 15 mL falcon tube. The column is kept upright and the cap loosened.
12. Following similar steps in the *Material Buffer Exchange* section, centrifuge the column device at 1000xG for 2 mins. The flow-through is discarded and the device was placed back into the same falcon tube.
13. 1 mL 1X PBS is added directly on top of the resin. The device is centrifuged at 1000xG for 2 mins and the flow-through was discarded. This step is repeated two more times for a total of 3 washes.
14. After the last wash step, the column is removed from the falcon tube. Keeping the column upright, the bottom of the column is blotted off with a Kimwipe and was transferred to a clean 15 mL falcon tube.
15. The biotinylated 8H4 Ab is applied directly on top of the resin. After 1 min, 40 µL of 1X PBS is applied as a stacker.
16. The device is centrifuged at 1000xG for 2 mins. The column is discarded and the flow-through is kept on ice.
17. The purified biotinylated 8H4 Ab solution is transferred into a clean 1.5 mL microtube, covered with foil and placed in the 4°C fridge. The final volume collected is measured using a pipette and recorded.

18. Use NanoDrop (Protein IgG concentration setting) to determine the concentration of the Ab. *Note: BCA can be used as an alternative to NanoDrop.*

****Note**

Calculations for Biotinylation

1. Calculate the concentration (mM) of the Sulfo-NHS-SS-Biotin to add to the reaction in order to obtain a specific molar excess. Typical challenge ratio is 20 Biotin: 1 molecule of protein

for a 20 molar excess. The 8H4 Ab has a concentration of 1.8mg/mL in 50µL solution. Antibodies in general are ~150 kDa or 150,000 mg/mmol.

Equation used:

$$Vol\ Ab\ \times\ Conc.Ab\ \times\ molar\ wt.Ab\ \times \frac{molar\ excess\ biotin}{moles\ of\ protein} =\ mmol\ Biotin$$

$$0.05\ mL \times \frac{1.8\ mg}{1\ mL} \times \frac{1\ mmol}{150{,}000\ mg} \times \frac{20\ mmol\ Bi}{1\ mmol} = 0.000012\ mmol\ Bi$$

2. To calculate the volume (in µL) of 10 mM Sulfo-NHS-SS-Biotin to add to the labeling reaction, where MW Biotin = 906.7 mg/mmol:

$$0.000012\ mmol\ Bi \times \frac{606.7mg}{1\ mmol} = 0.0072804\ mg\ Bi$$

$$0.0072804\ mg\ Bi \times \frac{1mL}{0.5mg} \times \frac{1000uL}{1mL} = \mathbf{14.6uL}\ of\ 10mM\ Biotin\ stock\ solution$$

Cross-Species PrP ELISA

Critical Equipment

Description	Manufacturer	Model Number	Broad ID
SpectraMax M5 Plate Reader	Molecular Devices, Inc.		101058

Critical materials, reagents, and supplies

Name	Manufacturer	Model #
Anti-PrP Ab EP1802Y	Abcam	ab52604
Biotin-8H4 detection antibody	Broad Institute	N/A
Recombinant mouse prion protein	Broad Institute	Mo PrP16

General materials, reagents, and supplies

Name	Manufacturer	Catalog #
TMB substrate	Cell Signaling Technology	7004P4
Stop solution	Cell Signaling Technology	7002L
CHAPS hydrate	Sigma	C9426
Milli-Q water	Millipore	N/A
Pierce High Sensitivity Streptavidin-HRP	Thermo Scientific	21130
96 Well Flat- Bottom Immuno Plate, MaxiSorp	Thermo Fisher Scientific	439454
0.22µm vacuum filter system	Corning	CLS431098
Bovine Serum Albumin	SeraCare Life Sciences	19K15A0018
1X PBS CSHL, pH 7.4	Broad Institute SQM	N/A
10% Tween-20 solution	Teknova	T0710
Seal, Clear Adhesive MicroAmp Film	Life Technologies	4306311

Reagent Preparation

- Wash buffer: 1X PBS with 0.1% Tween-20
  Dilute 10% Tween-20 to 0.1% in 1X PBS. Example: 990mL 1X PBS + 10mL 10% Tween-20. Store at RT for up to 2 months

- Assay buffer: 1X PBS with 5% BSA and 0.05% Tween-20
  Dilute the required amount of BSA and 10% Tween-20 in 1X PBS. Mix thoroughly. Example: 25 g BSA + ~400mL 1X PBS + 2.5 mL 10% Tween-20. Add 1X PBS to a final volume of 500mL. Filter through a 0.22 µm vacuum filter. Store at 4°C for up to 1 month.

- Standards
  Prepare high standard (Std01) by diluting stock MoPrP16 to 5ng/mL is assay buffer.
  Make 6 serial dilutions to produce the concentrations 2, 0.8, 0.32, 0.128, 0.0512, and 0.02048 ng/mL (Std02-07).
  The low standard (Std08) is neat assay buffer.
  Make a standard curve fresh from frozen, undiluted rPrP stock every time.

- QC Samples

The QC samples used are: Mo Pos Hi QC, Mo Pos Mid QC, Mo Pos Lo QC, and Mo Neg QC. The QCs are stored at -80°C and are in 40 µL aliquots.

Procedure

1. Prepare capture Ab solution by diluting capture antibody EP1802Y to 2.0 µg/mL in PBS. Vortex briefly to mix. Prepare enough Capture Ab solution to add 100 µL to each plate well plus a 10% excess. Seal the plate and store overnight at 4°C.
2. Wash plate 3x with 300 µL Wash buffer per well. Tap dry.
3. Block plate by adding 250 µL Assay buffer per well. Seal and incubate at RT for 1-3 hours.
4. Wash plate 3x with 300 µL Wash buffer per well. Tap dry.
5. While the plate is blocking, dilute standards, QCs, and samples in assay buffer and add 100 µL of each to the plate per plate map. Pipette up and down to mix. Seal and incubate at RT for 60-75 minutes.
6. Wash plate 3x with 300 µL Wash buffer per well. Tap dry.
7. Prepare detection Ab solution by diluting biotin-labeled 8H4 detection antibody to 0.25 µg/mL in assay buffer. Vortex briefly to mix. Prepare enough detection Ab solution to add 100 µL to each plate well plus a 10% excess. Seal the plate and incubate at RT for 60-75 minutes.
8. Wash plate 3x with 300 µL Wash buffer per well.
9. Prepare streptavidin-HRP solution by diluting streptavidin-HRP to 24.69 ng/mL in assay buffer. Vortex briefly to mix. Prepare enough Streptavidin-HRP solution to add 100 µL to each plate well plus a 10% excess. Seal and incubate at RT for 20-30 minutes. *(**Note: full 30 minutes recommended, otherwise the plate may not reach ~0.8 OD in the 30-minute time from during the TMB incubation step.)*
10. Wash plate 3x with 300 µL Wash buffer per well
11. Add 100 µL per well of TMB to plate. TMB solution should come to RT before using. Cover and incubate at RT until Std01 (5ng/mL) reaches ~0.8 OD. Pre-read plate at 605nm. If Std01 does not reach this OD within 30 minutes stop plate and read.
12. Add 100 µL per well of Stop solution to plate. Stop solution should come to RT before using. Mix well on plate reader briefly and read at 450nm and 630nm.