Supplementary material

Optimized filter trap assay for the detection of aggregated alpha-synuclein in brain samples; step by step standard operating procedure

1. Take 10 mg of frozen brain tissue and place the sample in a 2 mL Lysing Matrix D tube (MP Biomedicals, Cat# 6913050, Illkirch, France)

2. Add 300µL of radioimmunoprecipitation assay (RIPA) buffer (Merck Millipore, Cat# 20-188, Molsheim, France) containing 2 mM orthovanadate (Sigma, Cat# S6505, Saint Quentin Fallavier, France), 1% (v/v) phosphatase inhibitor cocktail III (Sigma, Cat# P0044) and a protease inhibitors cocktail (Roche, Cat# 14424700, Meylan, France) at 4°C and lyse the samples with the "Precellys 24" tissue homogenizer (Bertin technologies, Cat# P000669-PR240-A, Montigny-le-Bretonneux, France)

3. Transfer the lysate to a 1.5 mL Eppendorf 3810X tube (Eppendorf, Cat# 003012515, Montesson, France) and keep on ice

4. Sonicate samples (1 min, 5sec on / 5 sec off, amplitude 70%); we use the VCX130 ultrasonic processor (Sonics and Materials, Newton, CT, USA)

5. Centrifuge at 10,000 x *g* for 5 min at 4°C, to remove debris

6. Collect the supernatant and determine protein concentration using the Pierce BCA Protein Assay Kit (ThermoFisher Scientific, Cat#23227, Saint Herblain, France); we use the Varioskan™ multimode microplate reader (ThermoFisher Scientific, Cat# VL0L00D0). Adjust protein concentration to 1 mg/mL

7. Add the reducing agents (NuPAGE™ Sample Reducing Agent 10X, ThermoFisher Scientific, Cat# NP0004)

8. Heat the samples for 5 min at 98°C, then keep the samples on ice before loading

9. Set up the Bio-Dot™ Microfiltration System (Bio-Rad, Cat# 1706545, Marne la Coquette, France):

- soak one 12.5 x 9 cm 0.45 µM pore size cellulose acetate membrane (Sterlitech, Cat#CA0453001, Kent, USA) in Tris-buffered saline (TBS, Sigma, Cat# T5912) for at least 5 minutes.
- soak two 12.5 x 9 cm Whatman filter papers in TBS for 10 seconds
- place these on the base of the Bio-Dot Microfiltration System, with the cellulose acetate membrane on top of the filter papers
- roll out any air bubbles using a glass pipette
- place the rubber seal and lid, insert and tighten the screws diagonally; connect the filtration pump. Add 150 µL of TBS to each well and apply full vacuum until the wells are empty (make sure that none of the wells are clogged)

10. Load 20 µg of samples per well and apply low vacuum until the wells are empty (should take 3 to 5 minutes)

11.Detect protein aggregates on cellulose acetate membrane

- incubate the membrane for one hour at room temperature in TBS with 0.1% (v/v) Tween-20 (Sigma, Cat# P1379) and 5% (w/v) non-fat dry milk; then overnight at 4°C in the same buffer with the primary antibodies, MJFR1 (Abcam, Cat# ab138501, rabbit monoclonal, 1:1,000) or EP1536Y (Abcam, Cat# ab51253, rabbit monoclonal, 1:500)
- wash the membrane 3 times for 5 min in TBS-Tween 0.1%
- detect bound antibodies with horseradish peroxidase-conjugated anti-rabbit (Life technologies, Cat# 31460, diluted 1:5,000) in TBS-5% non-fat dry milk for one hour at room temperature
- wash the membrane 3 times for 5 min in TBS-Tween 0.1%
- apply 1 mL of SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Life technologies, Cat# 34580) and develop