

Sandwich ELISA for alpha-synuclein

*Antibody pairing determined based on sample type and should be optimized

Day 1

Coating

- Coat 384-well plate with primary antibody
 - Coating buffer: 200mM NaHCO₃ with 0.02% sodium azide (pH9.6)
 - 1^o antibody most used is syn1 at 1:500 dilution (BD 610787)
- Add 25uL per well (avoiding outer wells)
- Incubate on rocker at 4^o overnight

Day 2

Blocking

- Make blocking buffer (PBS+2.5% gelatin, 0.025% tween)
- Wash plate 3X using 1xPBS-T in plate washer
- Blot on paper towel
- Add 80ul per well of blocking buffer
- Incubate at 37^o for 1 hour
- During incubation, prepare standards and samples on ice, dilute in blocking buffer (BB)

Samples

- Standards – use full length recombinant alpha-synuclein. Stock aliquoted upon opening.
 - Make serial dilutions in blocking buffer, starting from 10ng/uL stock
- Dilute samples in blocking buffer
- Wash plate 3X, blot on paper towel
- Add 25ul per well of standards and samples (in duplicate)
- Incubate at room temperature for 2 hours

Detection antibody

- Bring blocking buffer to room temperature before use
- Dilute detection antibody in blocking buffer
 - 2^o antibody most used is biotinylated-hSA4, at 1:200 dilution (commercially MJFR1 ab138501; biotinylated in-lab)
- Wash plate 3X, blot on paper towel
- Add 25ul per well of detection antibody
- Incubate at 37^o for 1 hour

Avidin-alkaline phosphatase

- Wash plate 5X, blot on paper towel
- Use avidin-alkaline phosphatase at 0.6ul/mL of blocking buffer
- Add 25ul per well of AAP
- Incubate at 37^o for 30 mins

Substrate

- Make pNPP – 1 of each tablet for 5mL ddH₂O (cover tube in aluminum, rock to dissolve)

- Wash plate 5X, blot on paper towel
- Add 25ul/well of pNPP, read plate immediately
 - Read absorbance at 405nm, every 2.5 minutes for 60 minutes
 - Use data from a read when the standards are linear