Aggregated aSyn Dot Blot assay

1. Normalize protein samples to equal concentrations between 100ng-1000ng / uL in PBS.
2. Carefully spot 1.5uL each sample onto dry nitrocellulose (0.45um pores) membrane
3. Allow moisture to dry for a minute or two
4. Block membrane in 5% skim milk in TBST
5. Wash membrane 1X in TBST
6. Apply anti-aggregated aSyn antibody in 5% skim milk in TBST (1:1000 for Abcam fibril.aggregate specific aSyn antibody: MJFR 14-6-4-2)
7. Incubate at RT for 1-2hrs, or overnight at 4C
8. Wash 3x with TBST
9. Incubate with anti-rabbit IgG (or appropriate secondary) at 1:1000 for 1-2hrs in TBST
10. Wash 3x with TBST
11. Develop with Biorad Chemiluminescent substrate, or similar

Proteinase K resistant aSyn histology

-Certainly can be modified for either DAB or Fluorescent staining, I have DAB protocol too-

1. Wash 50um brain sections 1x in PBS
2. Treat with Protinase K (NEB, 1:4000, stock at 20mg/mL) for 10min at RT
3. Wash 2x with IHC buffer (3% NGS, 0.3% TX100, in PBS)
4. Block with Mouse-on-Mouse kit (Vector Lab, PK-2200, just first step in instructions) for 1hr
5. Wash 2x with IHC buffer
6. Incubate overnight at 4C with primary antibody 1:250 – 1:1000 (anti-synuclein BD Biosci 610787)
7. Wash 3x with PBS
8. Incubate with desired secondary (anti-Mouse IgG AF488 is my choice at 1:1000)
9. Wash 3x with PBS
10. Stain with Nissl and Mount with Profade with DAPI