Direct ELISA

1. Dilute 100-**500** ng of protein of interest (e.g. α-synuclein) in Takeda buffer per well and add 30 µL total liquid per well to 384-well Nunc Maxisorp plate.
2. Seal with removable clear adhesive cover
3. Centrifuge the plate at 1000 x g for 1 min to pull down protein onto the plate. Leave for 4h at 37°C or o/n at 4°C.
4. Use the plate washer with 5x with 100 µL PBST.
5. Block with 100 µL Blockace per well. Fill wells from the bottom, being sure to avoid leaving any bubbles in the wells.
6. Seal with removable clear adhesive cover and leave for 4h at 37°C or o/n at 4°C.
   *At this point, plates can be stored for up to 1 month at 4°C if there is preservative in the buffer.
7. Use the plate washer with 5x with 100 µL PBST.
8. Use C buffer to dilute reporter antibody. Vortex immediately before pipetting.
9. Using multichannel, fill 91, dispense 30 µL three times.
10. Seal with removable clear adhesive cover and centrifuge plate at 1000 x g for 1 min.
11. Incubate for 4h at 37°C or o/n at 4°C
12. Use C buffer to dilute HRP-conjugated secondary reporter antibody.
13. Add 30 µL per well. For goat-anti-mouse/rabbit use at 1:5-20K
14. Seal with removable clear adhesive cover and centrifuge plate at 1000 x g for 1 min.
15. Incubate for 1h at 37°C.
16. Use the plate washer with 5x with 100 µL PBST.
17. Add 30 µL TMB reagent per well.
18. Develop for 10-30 min.
19. Quench using 30 µL 10% phosphoric acid per well.
20. Read plate on the Spectramax or similar plate reader. 384-495 nm for unquenched reactions, 450 nm for quenched reactions.