

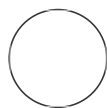


DEC 01, 2023

Direct ELISA

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ABSTRACT

This protocol details Direct ELISA.

ATTACHMENTS

[911-2362.pdf](#)

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Protocol status: Working
We use this protocol and it's working

Created: Nov 29, 2023

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Keywords: ASAPCRN

Funders



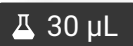
Acknowledgement:

Aligning Science Across
Parkinson's


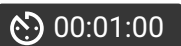

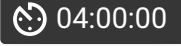
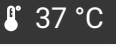
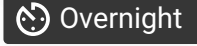

Grant ID: ASAP-020616

Direct ELISA


13h 33m

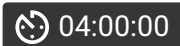
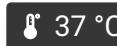


- 1 Dilute  100 ng -  500 ng of protein of interest (e.g. α -synuclein) in Takeda buffer per well and add  30 μ L total liquid per well to 384-well Nunc Maxisorp plate.

- 2 Seal with removable clear adhesive cover.

- 3 Centrifuge the plate at  1000 x g for  00:01:00 to pull down protein onto the plate. Leave for  4h 1m  04:00:00 at  37 °C or  Overnight at  4 °C .

- 4 Use the plate washer with 5x with  100 μ L PBST.

- 5 Block with  100 μ L Blockace per well. Fill wells from the bottom, being sure to avoid leaving any bubbles in the wells.

- 6 Seal with a removable clear adhesive cover and leave for  04:00:00 at  37 °C or  Overnight  4h

at 4 °C .

Note

At this point, plates can be stored for up to 1 month at 4 °C if there is a 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 00:01:00 . 1m





11 Incubate for 04:00:00 at 37 °C or Overnight at 4 °C . 4h



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  00:01:00 . 1m



15 Incubate for  01:00:00 at  37 $^{\circ}\text{C}$. 1h



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.