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Direct ELISA

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Protocol status: Working

We use this protocol and it's working

Created: November 29, 2023

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Abstract

This protocol details Direct ELISA.

Attachments



[911-2362.pdf](#)





169KB

Troubleshooting











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  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 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 μ L 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  1m 
- 11 Incubate for  04:00:00 at  37 °C or  Overnight 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  1m 
- 15 Incubate for  01:00:00 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.