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We use this protocol and it's working

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Unconventional secretion of alpha-synucein mediated by palmitoylated DNAJC5 oligomers

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ABSTRACT

This protocol, carried out according to manufacturer's instructions uses a classical ELISA experimental design with sensitive electrochemical detection to provide measure of -synuclein concentration in the conditioned media of cultured iPSC-derived human cells.

GUIDELINES

All aspects of the protocol for the MSD assay are carried out according to manufacturers instructions: summarised below. All reagents are included in the U-PLEX Human -synuclein Kit unless otherwise stated.

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MATERIALS

U-PLEX Human □- synuclein Kit - (Meso Scale Discovery (MSD), K151WKK-1)
Phosphate-buffered saline (PBS 1x), sterile filtered, - (Thermofisher, SKU#J61196.AP)
Tween-20 - (Sigma -Aldrich, SKU#P1379)
TritonTM X-100 - (Sigma -Aldrich, SKU#11332481001)
Sodium deoxycholate - (Sigma -Aldrich, SKU#D6750)
SDS (20% Solution) - (Santa Cruz Biotechnology, SKU#SC-24950)
EDTA - (Sigma -Aldrich, SKU#E9884)
NaCl - (Sigma -Aldrich, SKU#S9888)
PierceTM BCA Protein Assay Kit - Thermofisher, SKU#23225)

Equipment:
MESO QuickPlex SQ120 - (Meso Scale Discovery (MSD), 1300170428908)

BEFORE START INSTRUCTIONS

Conditioned media used in this assay can come from any human cell line expressing □-synuclein though volumes and culture conditions have been optimised for iPSC-derived dopamine neurons differentiated according to the following protocol: dx.doi.org/10.17504/protocols.io.q26g7y1jqgwz/v1 and plated at 100,000 cells per well in full area 96-well plates and cultured in 100 µL of media.

Harvesting media and cell lysates

1 Harvest conditioned media from cells grown in a full-area 96 well plate, being careful not to disturb the cell monolayer. Add 30 uL RIPA lysis buffer (0.5% Triton X-100, 10 mM Tris/HCl, pH 8.0, 1 mM EDTA, 0.5 mM EDTA,0.1% sodium dodecyl sulfate, 0.02% sodium deoxycholate, and (140 mM NaCl) to the cells remaining in each well and incubate on ice for 30 mins.

2 Centrifuge the harvested media 10 mins at 1000 xg to pellet floating cells. Collect the supernatant to use in the assay.

3 Use a pipette to homogenise the cells in lysis buffer and then determine the total protein concentration of the lysate using the Pierce BCA Protein Assay Kit according to manufactures instructions.

Measuring □-synuclein in conditioned media

4 Dilute the biotinylated capture anti-body 1:50 in Diluent 49 and add 25 µL to each well of the provided plate. Seal the plate and incubate at room temperature for 1hr with agitation

5 During the incubation prepare the standard curve by serial dilution of the calibrator sample in Diluent 49. For the highest concentration standard add 15 µL of calibrator to 285 µL to give a 10,000 pg/ml solution and then serially dilute 1:4 to give a range of standards between 2.44 pg/ml and 10,000 pg/ml. Use Diluent 49 alone as a blank.

6 Wash the plate 3 times to remove capture antibody with wash buffer (PBS containing 0.05% Tween-20, prepare in advance)

7 Dilute detection antibody 1:50 in Diluent 49 and add 25 µL to each well.

8 Add 25 µL of conditioned media supernatant (prepared as described above) or □-synuclein standard to each well. Seal the plate an incubate for 2 hrs at room temperature with agitation.

9 Repeat step 3 to remove unbound sample and detection antibody

10 Add 150 µL of Read Buffer and measure electrochemiluminescence on the MESO QuickPlex SQ 120 immediately

Data Processing

11 Using the calibrator samples interpolate the □-synuclein concentration by fitting a polynomial in the form y = ax2 + bx +c

12 Multiply the concentration of □-synuclein determined from the MSD assay by the total volume of media in each well (100 µL) to obtain the total value in pico-grams released by the cells.

13 Normalised release for difference in plating density/cell number by dividing by the total protein values obtain by BCA