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

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Cytokine profiling analysis on conditioned medium of human neurons using Luminex multiplex assay

Ching-Chieh Chou[1], Judith Frydman[1]

[1]Department of Biology, Stanford University

Ching-Chieh Chou
Stanford University

ABSTRACT

This protocol is used to identify secreted inflammatory factors by cytokine profiling of the conditioned medium from human transdifferentiated neurons of healthy donors and AD patients.

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Sample collection

1 At post-induction day 35 to 38, human transdifferentiated neurons undergo the final round of medium change.

2 Two days after medium change, a minimum of 200 µL of cell culture medium per sample is collected to run duplicate wells without dilution.

3 Conditioned medium is centrifuged at 10,000 X G for 10 min at room temperature to pellet out particulates.

4 Supernatant is collected and immediately stored at −80°C.

5 Cells are then trypsinized by 0.05% Trypsin-EDTA for 5 min and centrifuged at 200 X G for 5 min. Decant the supernatant and resuspend cells in a small aliquot of culture medium.

6 Determine cell number for each sample by a hemocytometer for normalization of cytokine levels.

Sample preparation and measurement

7 Thaw samples on ice prior to loading a 96-well filter plate for assay.

8 During this time, prepare Standards by rehydrating the lyophilized standard vial with Assay Buffer. Vortex and incubate on ice for 25 min.

9 Perform serial dilutions in Assay Buffer and Standards must be used within 1 hr.

10 The 96-well filter plate is incubated with 120 µL/well of Reading Buffer for 10 min at room temperature on an orbital shaker at 500-600 rpm.

11 Completely remove Reading Buffer. Add 25 µL/well of samples or Standards and 25 µL/well of Assay Buffer.

12 Incubate with 50 µL/well of Antibody Beads for 2 hr at room temperature on an orbital shaker at 500-600 rpm. Then, transfer the plate to a refrigerator for overnight incubation at 4°C.

13 Next day, take out the plate from the refrigerator and stay at room temperature for 30 min without shaking.

14 Remove solutions and wash the plate with Wash Buffer for 3 times.

15 Add Detection Antibody with 25 µL/well and incubate for 2 hr at room temperature on an orbital shaker at 500-600 rpm.

16 Remove solutions and rinse the plate with Wash buffer for 3 times.

17 Add Streptavidin-PE with 50 µL/well and incubate for 40 min at room temperature on an orbital shaker at 500-600 rpm.

18 Remove solutions and wash the plate with Wash buffer for 3 times.

19 Add Reading Buffer with 120 µL/well and incubate for 5 min at room temperature on an orbital shaker at 500-600 rpm.

20 Read the plate on Luminex instruments following the manufacturer's instructions.