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

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We use this protocol and it's working

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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.

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 μ L 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 μ L/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 μ L/well of samples or Standards and 25 μ L/well of Assay Buffer.
- 12 Incubate with 50 μ L/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 μ L/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 μ L/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 μ L/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.