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iPSC derived microglia (hMG) culture

In 2 collections

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Lucas Blasco-Agell[1,2], Veronica Testa[1,2], Jara Montero-Muñoz[1,2], Antonella Consiglio[1,2], Miquel Vila[3]

[1]Department of Pathology and Experimental Therapeutics, Bellvitge University Hospital- IDIBELL, 08908 Hospitalet de Llobregat, Spain;
[2]Institute of Biomedicine of the University of Barcelona (IBUB), Carrer Baldiri Reixac 15-21, Barcelona 08028, Spain.;
[3]VHIR-CIBERNED-ASAP

Vilalab Public

Miquel Vila
VHIR-CIBERNED-ASAP

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We use this protocol and it's working

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Abstract

iPSC derived microglia (hMG) culture

1 Single iPSC colonies are manually passaged onto fresh Matrigel-coated 6 cm^2 plastic plate.

2 After 2-4 days, protocol starts by supplementing mTeSRTM1 medium with 80 ng/mL of Bone Morphogenetic Protein (BMP)-4 every day from day 0 to day 4.

3 From day 5, SP34 medium is employed, which consisted of StemProTM-34 SFM (Gibco™) with 1% of P/S and 1% of Ultraglutamine (Glut; Lonza). For two days, SP34 medium is supplemented with 80 ng/ml of VEGF, 100 ng/ml of Stem Cell Factor (SCF) and 25 ng/ml of Fibroblast Growth Factor (FGF)-2.

4 After day 7 until day 14, SP34 is supplemented with 50 ng/ml of Fms-like tyrosine kinase 3-Ligand (Flt3-L), 50 ng/ml of IL-3, 50 ng/ml of SCF, 5 ng/ml of Trombopoietin (TPO) and 50 ng/ml of M-CSF, changing the medium at day 10.

5 The last step consisted on the addition to SP34 of Flt3-L, M-CSF and 25 ng/ml of GM-CSF from day 14, changing the medium every 3-4 days. At day 35, floating hMG progenitors are collected from the culture's supernatant and passed through a 70 µm Filcon™ Syringe-Type nylon mesh (BD Biosciences) to ensure a single-cell suspension and to remove cell clumps.

6 Cells are counted, centrifuged at 300x*g* for 10 minutes and cultured in Roswell Park Memorial Institute (RPMI) 1640 Medium (Gibco™) with 1% of P/S and 1% of Glut, supplemented by M-CSF and 50 ng/ml
IL-34.

7 Medium is changed with fresh factors every 2-3 days. hMG progenitors are plated onto Matrigel-coated glass coverslips in 24-well plates (25,000 cells/well), on uncoated 96-well plates (10,000 cells/well) or 6-well plates (200,000 cells/well) depending on the experiment.

8 hMG mature cells are cultured for one week, before being used for experiments. For treatments, 100 ng/mL of LPS or 5 ug/mL of NM are added for a total of 24 hours.