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iPSC-derived vmDAn

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We use this protocol and it's working

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Abstract

iPSC-derived vmDAn

1 iPSC medium is changed to Serum Replacement Medium (SRM), which is based on KO-DMEM with
15% of KO Serum Replacement (KO-SR; Gibco™), 1 % of P/S, 1% of Glut, 1% of Non-Essential Amino Acids (NEAA; Lonza), and 10 µM of β-Mercaptoethanol (BME; Gibco™).

2 The first day, SRM are supplemented with 100 nM of LDN193189 hydrochloride (LDN; Sigma-Aldrich) and 10 µM of SB 431542 (SB; Tocris Bioscience). From there and during all the differentiation, cells are maintained under hypoxic conditions (37 ºC Temperature, 5% CO_2 and 5% O_2).

3 From day 1 to day 5, SRM medium is supplemented with same concentrations of LDN and SB and adding 1 µM of Smoothened Agonist (SAG; Merck Millipore), 2 µM of Purmorphamine (Tocris Bioscience), and 100 ng/mL of FGF-8. From day 3 onwards, 3 µM of StemMACS™ CHIR99021 (CHIR; Miltenyi Biotec) are also added.

4 From day 5, medium is progressively switched from SRM to N_2, which consists of Neurobasal™ Medium (Gibco™), 1x N_2 supplement (Gibco™), 1% of P/S and 1% of Glut. On day 11, medium was changed with N_2 supplemented with LDN and CHIR and cells could be either expanded as FP progenitors or matured to vmDAn.

5 At day 11, Cells were maintained in N_2 supplemented with LDN and CHIR, until reaching 90% of confluency within the plate. To differentiate FP progenitors into vmDAn, medium is changed to B27-vitA, which contained Neurobasal™, 2x B27™- vitamin A (Gibco™), 1% of P/S and 1% of Glut. Every two days, medium is fully changed with B27 supplemented with 20 ng/mL of BDNF, 20 ng/mL of Glial cell-line Derived Neurotrophic Factor (GDNF), 1 ng/mL of TGF-β3, 10 µM of Deoxyadenosine Triphosphate (dAPT; Calbiochem), 0.5 mM of Dibutyryl cyclic-Adenosine Monophosphate sodium salt (cAMP; Sigma-Aldrich) and 0.2 mM of L-Ascorbic Acid (AA; Sigma-Aldrich).

6 On day 20, cells are detached with Accutase (Merck Millipore), counted and centrifuged at 300x*g* for 5 minutes. Cells are plated on 6-well or glass coverslips in 24-well plastic plates with a triple-coating of 15 µg/mL Poly- L-Ornithine solution (Sigma-Aldrich), 3 µg/mL of Laminin (Sigma-Aldrich) and 2 µg/mL of Fibronectin (Sigma-Aldrich) to a density of 100,000 cells/cm2.

7 From day 20 onwards, half of B27 with factors is changed every 3-4 days until vmDAn are used for experiments.