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Forebrain neural progenitor cells (fbNPCs) differentiation from hiPSCs (dual SMAD inhibition)

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We use this protocol and it's working

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Abstract

This protocol described how to differentiate hiPSCs to forebrain neural progenitor cells using dual SMAD inhibition

hiPSCs dissociation

15m

1 Human induced pluripotent stem cells (hiPSCs) are rinsed once with DPBS (GIBCO) and dissociated when 70-90% confluent with 0.5 mM EDTA (75 ml/cm2; GIBCO) at 37 °C for 00:07:00

7m

2 Dissociated cells are then carefully washed away from the wells and collected in **wash medium** (9.5 mL DMEM/F-12 (31330-038; GIBCO) and 0.5 mL knockout serum replacement (GIBCO)).

3 The cells are then centrifuged at 400 x g for 00:05:00 and resuspended in iPS brew medium (StemMACS iPS-Brew XF and 0.5% penicillin/streptomycin (GIBCO)).

5m

hiPSCs differentiation into fbNPCs via dual SMAD inhibition

2w

4 The protocol is based on the one described in **Nolbrant et al., 2017** and **Grassi et al., 2019**.

Day 0 of differentiation. The resuspended cells are then plated on LN111-coated (1.14µg/cm2; Biolamina) coated Nunc multidishes at a density of 10000 cells/cm^2 and grown in **N2 medium** (1:1 DMEM/F-12 (21331020; GIBCO) and Neurobasal (21103049; GIBCO) supplemented with 1% N2 (GIBCO), 2 mM L-glutamine (GIBCO), and 0.2% penicillin/streptomycin). 10 µM of Y27632 (Rock inhibitor; Miltenyi) are also added to the cells when plating. Finally, 10 µM SB431542 (Axon) and 100 ng/ml noggin (Miltenyi) for dual SMAD inhibition are supplemented to the media.

5 The media, supplemented with dual SMAD inhibitors, is changed every 2-3 days until *day 9 of differentiation*. The amount of media is slightly increased at every feeding as the cells' confluency increases.

6 *Day 9 of differentiation.* The cells are fed with N2 media without dual SMAD inhibitors.

7 *Day 11 of differentiation.* The cells are dissociated, centrifuged, and resuspended as described above (hiPSCs dissociation SECTION). They are then replated on LN111-coated Nunc multidishes at a density of 800000 cells/cm^2 in **B27 medium** (Neurobasal supplemented with 1% B27 without vitamin A (GIBCO), 2 mM L-glutamine and 0.2% penicillin/streptomycin Y27632 (10 µM), BDNF (20 ng/ml; R&D), and L-

ascorbic acid (0.2 mM; Sigma)). 10 µM of Y27632 are also added. The cells are kept in the same medium until *day 14 of differentiation*, when they are harvested for downstream analyses (e.g. bulk RNA sequencing).