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Human iPSC-derived MgBr assembloids

In 1 collection

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We use this protocol and it's working

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Abstract

Human iPSC-derived MgBr assembloids

1 Seed 9,000 iPSCs into each well of a Nunclon™ Sphera™ 96-well microplate (Cat. number 174925; Thermo Fisher Scientific) to generate embryoid bodies (EBs).

2 Culture EBs from day 0 to day 5 in dual SMAD inhibition medium consisting of mTeSR™ Plus supplemented daily with 1% NEAAs, 1% GlutaMAX, 100 µM β-mercaptoethanol, 200 nM LDN 193189 hydrochloride (LDN), 10 µM SB, and 50 µM Y-27632 (ROCK inhibitor).

3 On day 6, switch to cortical induction medium consisting of 1:1 DMEM-Ham's F-12 and Neurobasal medium supplemented with 1% NEAAs, 1% GlutaMAX, 1% N2, 1% B27 without vitamin A, 100 µM β-mercaptoethanol, 20 ng/mL FGF2, and 20 ng/mL EGF.

4 On day 7, embed EBs in Matrigel at a 3:2 ratio of Matrigel to medium to form the embedding mixture.

5 Wash EBs with fresh medium, embed in Matrigel in a 6-well ultralow-attachment plate, and incubate the Matrigel–EB mixture for 30 minutes at 37°C.

6 Maintain organoids in cortical induction medium until day 14.

7 From days 14 to 30, maintain organoids in differentiation medium consisting of 100% Neurobasal medium supplemented with 1% NEAAs, 1% GlutaMAX, 1% N2, 1% B27 without vitamin A, 1% PS, 100 µM β-mercaptoethanol, 2.5 µg/mL insulin, 200 µM ascorbic acid, 20 ng/mL BDNF, and 1 mM dcAMP.

8 On day 20, dissociate Matrigel from organoids and place plates on an orbital shaker to increase oxygenation and improve medium distribution.

9 On day 30, transfer individual brain organoids into wells of a Nunclon™ Sphera™ 96-well microplate to generate iPSC-derived MgBr assembloids.

10 On day 10 of in vitro differentiation, seed 2 × 10^5 predifferentiated iPSC-derived microglia on top of each organoid in a final volume of 200 µL assembloid maturation medium.

11 Prepare assembloid maturation medium with 100% Neurobasal medium supplemented with 1% NEAAs, 1% GlutaMAX, 1% N2, 1% B27, 1% PS, 100 µM β-mercaptoethanol, 20 ng/mL BDNF, 1 mM dcAMP, 10 ng/mL GDNF, 100 ng/mL IL-34, 100 ng/mL M-CSF, and 10 ng/mL GM-CSF.

12 Change maturation medium twice daily for 3 days without removing microglia in suspension.

13 Transfer assembloids to a 6-well ultralow-attachment plate after 3 days.

14 After 2 additional days, place MgBr assembloids on an orbital shaker and maintain in maturation medium until day 45 (15 DPI) or day 65 (35 DPI) of differentiation.