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Working

Neural aggregate formation

In 1 collection

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Neurodegeneration Method Development Community

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PDF

IPSC CORTICAL DIFFERENTIATION 022017.pdf

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

This protocol is part of the IPSC CORTICAL DIFFERENTIATION collection.

This method should be performed using sterile technique.

MATERIALS TEXT

Please refer to the attached full manuscipt for requried materials.

SAFETY WARNINGS

Please refer to the SDS (Safety Data Sheet) for information about hazards, and to obtain advice on safety precautions.

1 Harvest iPSCs for neural aggregate formation when iPSCs have reached 75- 85% confluency. Aspirate medium and rinse with **2 ml** of DPBS.

2 Add **1 ml** of Accutase. Incubate at **37 °C** for **00:05:00** . Gently tap plate to dislodge cells.

3 Dilute Accutase with **4 ml** of DMEM/F12 medium and collect cell suspension in 15ml conical tube.

4 Centrifuge cells at 750 rpm for **00:03:00** . Then carefully aspirate medium from iPSC pellet.

5 Add **3 ml** of neural induction medium to iPSC pellet. Using a hemacytometer, count iPSCs. Adjust volume of iPSC suspension to 450-650,000 cells/mL using neural induction medium supplemented Rock inhibitor (10 µM final).

6 Add **100 µl** of iPSC suspension per well to a v-bottom 96-well plate.

7 Centrifuge plate at 750 rpm for **00:03:00** to sediment iPSC into spheres.

8 Incubate cells at **37 °C** , 5% CO2 and 95% humidified chamber for **24:00:00** . After 24 hrs, carefully remove all medium from well and replace with **100 µl** per well of Neural Induction Medium.

Do not disturb or break apart spheres. The spheres are very delicate at this stage.

9 Incubate neurospheres in 96 well plate for **96:00:00** . Perform half volume medium changes daily (removed **50 µl** and replace with **50 µl**).