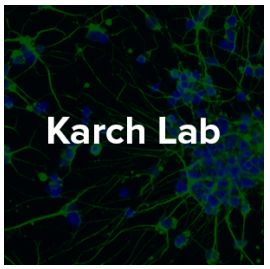
Feb 26, 2019

Neural aggregate formation



In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.x84fryw

Celeste M M. Karch[1], Rita Martinez[1], Jacob Marsh[1]

[1]Washington University in St Louis

Neurodegeneration Method Development Community
Tech. support email: **ndcn-help@chanzuckerberg.com**

Celeste M M. Karch
Washington University in St Louis

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.x84fryw

Protocol Citation: Celeste M M. Karch, Rita Martinez, Jacob Marsh 2019. Neural aggregate formation. **protocols.io https://dx.doi.org/10.17504/protocols.io.x84fryw**

Protocol status: Working
We use this protocol and it's working

Created: February 17, 2019

Last Modified: February 26, 2019

Protocol Integer ID: 20476

Attachments

PDF

IPSC CORTICAL DIFFER...

179KB

Guidelines

This protocol is part of the **IPSC CORTICAL DIFFERENTIATION** collection.

This method should be performed using sterile technique.

Materials

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.

Note

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).