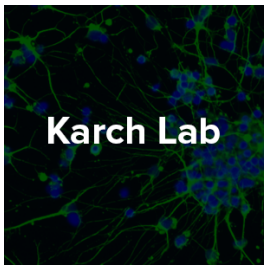
Feb 26, 2019

Neural progenitor expansion



In 1 collection

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

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Attachments

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 Using the pre-coated PLO-laminin plate, aspirate laminin from 2-3 wells. Remove supernatant from 15 mL conical tube containing 2 wells of neural rosette clusters. See protocol below.

Protocol

NAME

Neural Rosette Formation and Selection

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PREVIEW

1.1 On Day 5 of neural aggregate formation, remove media (by pipetting) and carefully wash spheres with 100 µL of pre-warmed DMEM/F12. Repeat 2 times.

Note

Do not break apart spheres. Neural spheres are very delicate at this stage. An alternative approach is to remove 50 µL of spent media and wash with 50 µL DMEM/F12. Add 50 µL fresh neural induction media. Transfer 100 µL of spheres and media to the new PLO/laminin-coated well. This approach will transfer more dead cells into the new well.

1.2 Remove the last wash and add 50 µL of neural induction media to each well.

1.3 Aspirate laminin from one well of the pre-coated plate. Using 200 µl sterile tips, carefully pipet up spheres from wells using 100 µL volume and transfer thirty-two spheres per well. Repeat above steps for the remaining wells. Incubate cells in 37 °C, 5% CO_2 and 95% humidified chamber and distribute evenly by making a “T” motion.

1.4 After 24:00:00, examine attached aggregates. Remove medium and replace with 2mls/well fresh neural induction medium daily.

Note

If some aggregates have not attached, carefully pipet out all medium and replace with 1ml/well fresh neural induction medium. Once 90-100% of aggregates attach, exchange medium daily with 2mls/well neural induction medium.

1.5 Monitor spheres daily under microscope for formation of neural rosette structures. Neural rosettes are ready to harvest when spheres have completely flattened and clusters are clearly visible (3-7 days after plating, line dependent).

1.6 Harvest neural rosettes by aspirating spent medium. Add 1 mL of pre-warmed DMEM/F12 to each well to remove unattached cells (repeat if necessary).

1.7 Add 1 mL of Neural Rosette Selection reagent to each well and incubate for up to 01:00:00 at 37 °C (check cells at 00:20:00 . Cells are typically collected after 30-45 min incubation. Look for rosette structure to be rounding up without the disturbance of other surrounding cells).

1.8 Carefully remove Neural Rosette Selection reagent with a pipet, being careful not to disturb rosette clusters. Add 1 mL DMEM/F12 to each well, then using a p1000 detach rosette clusters by rinsing over them.

1.9 Transfer rosette material from 1 well into a 15 mL conical tube for cryopreservation of neural rosettes and from 2 wells into a separate 15 mL conical tube for neural progenitor expansion. Do not triturate clusters.

Note

To maintain a pure culture, it is best to leave some rosettes behind rather than collect all of the rosettes and additional cells.

1.10 Centrifuge rosette clusters at 750 rpm for 00:03:00 .

2 Add appropriate amount of neural induction media (NIM) to achieve a final volume of 2 mL per well. Pipet clusters up and down breaking them into 1/4 or 1/5 the size of the rosette cluster.

3 Add 2 mL of neural rosette cluster suspension to each well and incubate at 37 °C , 5% CO_2 and 95% humidified chamber for 24:00:00 .

4 Examine adherent cells under microscope, aspirate off medium and replace with 2 mL of fresh neural induction medium daily for 3-5 days or until they reach ~80% confluent.

5 Make 12 mL of DMEM/F12 supplemented with 10% FBS by adding 2 mL of FBS to 10 mL of DMEM/F12 to inhibit trypsin activity (termed “Complete Media”).

6 Remove medium and rinse cells with 1 mL/well of DMEM/F12 .

7 Harvest cells by adding 1 mL/well of 0.05% trypsin and incubate at 37 °C for 00:03:00 .

8 Inhibit trypsin activity by adding 4 mL of DMEM/F12 supplemented with 10% FBS to each well.

9 Collect cells in 15mL conical tube. Centrifuge at 750 rpm for 00:03:00 .

Note

To minimize bubbles and increase cell yield, add 1mL Complete Media to 15mL conical tube. After trypsinization, add 3mL DMEM/F12 to collect cells and transfer to 15mL conical tube.

10 Aspirate supernatant and resuspend in 12 mL of NIM, pipet at least 3 times to break up large clumps.

Note

Small clumps and single cells are acceptable to passage.

11 Passage cells to pre-coated PLO-lamin plates (see above) by adding 2 mLs/well of suspended cells for a total of 6 wells.

12 Feed with 2 mL of fresh NIM daily until they reach 85-95% confluency.

13 **IMPORTANT:** NPCs are best maintained by passaging at a 1:3 dilution. Passaging cells that are more dilute will result in slow growth. Cells will typically become confluent after 2-4 days when plated as a 1:3 dilution. If NPCs are observed to slow in their growth, passage cells and replate without dilution.