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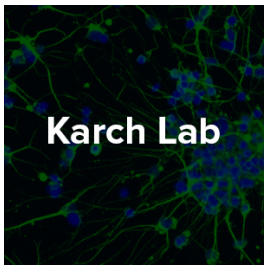
Preparation of feeder-free iPSCs culture



In 1 collection

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

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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 Coat appropriate sized tissue culture dish with Matrigel. Swirl plate to distribute evenly across surface of dish.

2 Allow Matrigel to set for 01:00:00 at room temperature or in a 37 °C humidified chamber.

Note

This will provide enough cells for a full 96 well plate of neural aggregates.

Note

We typically thaw 1 vial of iPSC into 1 well of a 6 well (1mL Matrigel per well). Volumes below are based on 6 well plates.

3 Prepare mTeSR1 feeder-free medium by aseptically adding 100 mL of thawed 5x supplement to 400 mL Basal Medium.

4 Add 5 mL penicillin/streptomycin if needed. Mix thoroughly and warm to room temperature.

5 Thaw iPSCs (1-2 × 10^6 cells/mL) in a 37 °C water bath.

6 Add 1 mL of thawed iPSC to 9 mL of DMEM/F12 in a 15mL conical tube (Do not mix). Centrifuge iPSC at 750 rpm for 00:03:00 .

7 Carefully aspirate medium from iPSC pellet. Add 2 mL of mTeSR1 supplemented with Rock inhibitor (10 µM final) - **avoid breaking up clumps of suspended cells.**

8 Aspirate Matrigel from tissue culture dish. Add 2 mL of suspended iPSCs. Place plate in 37 °C, 5% CO_2 and 95% humidified chamber, making a “T” motion to ensure even distribution of cells.

9 Change medium with 2 mL of fresh mTesR1 medium daily.

Note

As iPSC become more confluent, increase mTesR to 3mL per well.