



Feb 27, 2019 Working

## **Neural Rosette Formation and Selection**

In 1 collection

Celeste Karch<sup>1</sup>, Rita Martinez<sup>1</sup>, Jacob Marsh<sup>1</sup>

<sup>1</sup>Washington University in St Louis

dx.doi.org/10.17504/protocols.io.x86frze

Celeste Karch

Neurodegeneration Method Development Community

Tech. support email: ndcn-help@chanzuckerberg.com

Washington University in St Louis



PROTOCOL STATUS

022017.pdf

## Working

We use this protocol in our group and it is working

**GUIDELINES** 

This protocol is part of the <a href="IPSC CORTICAL DIFFERENTIATION">IPSC CORTICAL DIFFERENTIATION</a> collection.

This method should be performed using sterile technique.

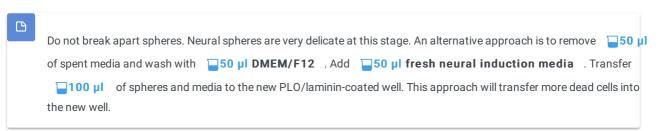
MATERIALS TEXT

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 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.



2 Remove the last wash and add 50 μl of neural induction media to each well.

- 3 Aspirate laminin from one well of the pre-coated plate. Using 200 μl sterile tips, carefully pipet up spheres from wells using uolume and transfer thirty-two spheres per well. Repeat above steps for the remaining wells. Incubate cells in 37 °C , 5% CO<sub>2</sub> and 95% humidified chamber and distribute evenly by making a "T" motion.
- 4 After (324:00:00), examine attached aggregates. Remove medium and replace with 2mls/well fresh neural induction medium daily.
  - 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.
- 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).
- 6 Harvest neural rosettes by aspirating spent medium. Add 11 ml of pre-warmed DMEM/F12 to each well to remove unattached cells (repeat if necessary).
- 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).
- 8 Carefully remove Neural Rosette Selection reagent with a pipet, being careful not to disturb rosette clusters. Add 11 ml DMEM/F12 to each well, then using a p1000 detach rosette clusters by rinsing over them.
- 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.
  - To maintain a pure culture, it is best to leave some rosettes behind rather than collect all of the rosettes and additional cells.
- Centrifuge rosette clusters at 750 rpm for **© 00:03:00** .

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited