FEB 27, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.x87frzn

Protocol Citation: Celeste M M. Karch, Rita Martinez, Jacob Marsh 2019. Neural rosette banking. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.x87frzn>

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

Created: Feb 17, 2019

Last Modified: Feb 27, 2019

PROTOCOL integer ID:
 20479

Neural rosette banking

In 1 collection

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ATTACHMENTS

[IPSC CORTICAL DIFFERENTIATION 022017.pdf](#)

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 manuscript for required materials.

SAFETY WARNINGS

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

- 1 Make 2x stock of serum-free neural freezing medium by adding 10 mL of sterile DMSO to 40 mL of sterile KOSR into a 50 ml conical tube. Mix by inverting 3-4 times. Store at 4 °C for up to 4 weeks.

- 2 Aspirate supernatant from 15mL conical tube containing 1 well of neural rosette clusters. See protocol below.

Protocol

NAME
Neural Rosette Formation and Selection

CREATED BY
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PREVIEW

- 2.1 On Day 5 of neural aggregate formation, remove media (by pipetting) and carefully wash spheres with 100 μ L 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 μ L of spent media and wash with 50 μ L DMEM/F12. Add 50 μ L fresh neural induction media. Transfer 100 μ L of spheres and media to the new PLO/laminin-coated well. This approach will transfer more dead cells into the new well.

- 2.2 Remove the last wash and add 50 μ L of neural induction media to each well.

- 2.3 Aspirate laminin from one well of the pre-coated plate. Using 200 μ L sterile tips, carefully pipet up spheres from wells using 100 μ L volume and transfer thirty-two spheres per well. Repeat above steps for the remaining wells. Incubate cells in 37 $^{\circ}$ C, 5% CO₂ and 95% humidified chamber and distribute evenly by making a “T” motion.

- 2.4 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.

2.5 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).

2.6 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).

2.7 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).

2.8 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.

2.9 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.

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

- 3 Add 1.5 mL of neural induction media supplemented with 1.5 mL of 2x neural freezing medium to the single well collection of neural rosette clusters. Gently mix solution and distribute 1 mL into sterile cryovials.
- 4 Store cryovials at -80 °C for at least 48:00:00 then transfer to liquid nitrogen for long-term storage.