NEUROMICS

E18 Primary Rat Neurospheres

Catalog Number: NS36100

Components : Approximately 1 x 10^6 cells
12 mls Culture Media.: Rat Neurosphere/Progenitor Proliferation Media (Neuromics' Catalog#: M36111).
Cell stored in **2mls Hibernate EB:** Hibernate E®/B27®/Glutamax™
5 mls Hibernate E-Ca:

Description: Live, unseparated, fresh from E18 rat cortex/hippocampus including subventricular zone. One order is from one brain which will provide over 1 million neurospheres. NNSph can be used for expansion as neurospheres with repeated passage or differentiation into neurons or astrocytes.

Shipping/Storage: Neurospheres are shipped refrigerated. Cells are stable for up to 1 weeks when stored at 4-8ºC. It is recommended to culture the cells as soon as possible after receiving cells according to Application Notes outlined below.

Application Notes

Materials Needed Not Provided

- Ultra Low Adhesion Wells (Corning)
- Papain (Worthington or Sigma P4762) for enzymatic dissociation
- Sterile pipette tips or sterile Pasteur pipette Sterile centrifuge tubes
- Centrifuge to operate at 200xg Water bath at 30°C Additional media:
    - Rat Neurosphere/Progenitor Proliferation Media (Neuromics' Catalog#: M36111).

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Store tissue at 4℃ until ready to use (note: use tissue right away for highest yield).

Preparations

1. Use Corning , 6-well ultra-low attachment plates (Fisher Scientific: 3471). No additional substrate is required.
2. Prepare cell dissociation solution by dissolving 6 mg sterile papain in 3 ml Hibernate E-Ca for a final working concentration of 2 mg/ml papain. Incubate for 10 min. at 30°C.
3. Fire polish the tip of a 9" salinized pasteur pipette to an opening of app. 0.5 mm.
4. Aliquot 20 µl Trypan Blue (Sigma T8154) into a 0.5 ml tube for step 9.

Dispersal (in a sterile hood)

1. With the salinized pasteur pipette, carefully transfer Hibernate EB (HEB) to a sterile tube (save for step 3) leaving the tissue with minimal HEB.
2. Add 2 ml of dissociation media to the tissue and incubate for 10 min. at 30°C. Gently swirl every 5 min.
3. Remove cell dissociation solution leaving the tissue at the bottom. Return the HEB medium from step 1.
4. With the salinized pasteur pipette, triturate tissue for about 1 min. (90% tissue dispersal) avoiding air bubbles.
5. Let the undispersed tissue settle for 1 min.
6. Transfer supernatant with cells to a new sterile 15 ml tube. Leave app. 5 µl of the HEB containing debris.
7. Spin 1100rpm (200 x G), 1 min. Discard supernatant leaving app. 5 µl of the HEB containing the pellet.
8. Disperse the pellet of cells (flick the bottom of the tube with a finger) and re-suspend the cells in 4 ml. of Neurosphere/Progenitor Proliferation Media
9. Aliquot 20 µl of cell solution into 0.5 ml. tube containing 20 µl Trypan Blue (1:2 dilution).
10. Count in hemacytometer: calculate cells/ml. Phase bright.

Cell Plating (at room temperature in s sterile hood)

1. Dilution cells in proliferation media and (2.0 ml/cm^2) and plate at 16,000 cells/10 cm^2 or desired concentration.
2. Incubate 37°C, 5% CO_2, 9% oxygen, 95 % humidity.
3. After 2-3 days neuroprogenitors are present.
4. Change ½ the proliferation media every 3-4 days. Equilibrated at 37° 5% CO_2.

Frequently Asked Questions

1. Why do I see clusters forming right away?

Seeding cells at 30,000/cm^2 will result in rapid cluster formation. Without attachment, they will stay as progenitors in Neuropro and grow into neurospheres that can be harvested in 4-7 days. Evaluation with immunostaining for nestin or other antibodies can begin after 4 days. To see clonal growth, you can plate the original sample or these neurospheres at limiting dilution of 50 cells/cm^2 of tissue culture plastic or ultra-low adhesion plastic in the same medium**.**

To see pluripotency, plate dissociated neurospheres at 5 to 15 thousand cells/cm2 on substrates coated with poly-d-lysine (50 ug/ml water).

2. Should I seed single cells to do the neurosphere assay?

No, a neurosphere contains a cluster of cells. You can fix them and stain as a cluster or dissociate them with papain or trypsin, count to determine yield and/or fix to evaluate individual cells**.**

3. The cells were single after seeding but they formed cluster today. The sizes of some of the clusters are very big. Is this the right sign?

Yes.

4. Should I observe the neurosphere formation to evaluate the cells' progenitor stage?

No, wait at least 4 days.

5. How do I distinguish the neurosphere and cluster?

You can pass the neurospheres onto low adhesion plastic or onto adhesive surface and see differentiation. As long as cells are on low-adhesion plastic, they will stay as progenitors.

6. Do I need to do immunostaining at this stage?

Wait at least 4 days.