NEUROMICS

E18 Primary Rat Neurospheres

Catalog Number: NS36100 **Components :**

- **Approximately 1 x 10^6 cells**
- **12 mls Culture Media.:** Neurobasal/B27-retinoic acid/glutamax/egf/hgf (the last two are growth factors).
- **5mls** Hibernate-Ca; 5 mls (storage and dissociation media)

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 2 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**
- 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
- General cell culture supplies (culture plates, coverslips, etc.)
    - *To see pluripotency, plate dissociated neurospheres at 5 to 15 thousand cells/cm2 on substrates coated with poly-d-lysine (50 ug/ml water).*

.

Preparation of Neurospheres

1. Store tissue at 4°C until ready to use.
2. When ready to plate, make up 2 mls of enzymatic solution in shipping media without B27 (Hibernate-Ca; 5 mls supplied) containing 4 mgs (2mgs/ml) of papain. Make sure to sterile filter solution with a 0.2 micron filter after adding papain if source of enzyme is not sterile
3. Transfer the 2 ml of medium from the tissue tube into a 15 ml screw cap sterile tube; be careful not to disturb or remove tissue from the original tube. Save, do not discard.
4. Add 2 ml of media made in step 2 (Hibernate-Ca containing 2mgs/ml of papain). Incubate for 30 minutes at 30°C.
5. Remove enzymatic solution, again careful not to disturb or remove tissue, add back 1 ml of media saved in 15 ml tube.
6. Using a 1 ml pipettor with a sterile blue plastic tip, or a silanized 9-inch Pasteur pipette with the tip barely fire polished (preferable), suck the tissue with the medium into the pipette and immediately dispense the contents back into the same container. Take care not to create bubbles. Repeat this tituration step about 10 times or until most all the cells are dispersed.
7. Let undispersed pieces settle by gravity for 1 min.
8. Transfer the dispersed cells (supernatant) into the 15 ml tube that contains the 1ml of media from Step 2, and gently mix the cells by swirling.
9. Spin the cells at 1,100 rpm (200xg) for 1 minute. Discard the supernatant while being careful not to remove any of the cells from the cell pellet.

10. Flick the tube a few times to loosen the cell pellet. Resuspend pellet in 1 ml of Neurobasal/B27-retinoic acid/glutamax/egf/hgf. Further dilute the cells with growth media to the desired culture density.

Neurosphere FAQs:

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/cm2 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.