NEUROMICS

E18 Primary Mouse Hippocampal Astrocytes

Catalog Number: PC36112 **Components :** ●**4 Cortical Pair Cord (2 mls)**
Approximately 1 x 10^6 cells/Cortical Pair
●**12 mls Culture Media-** Neurobasal/10% Serum/3 mM glutamine; **5 mls Hibernate-Ca**

Description: C57 Primary Mouse Hippocampal Cells are live neurons isolated from micro-surgically dissected regions of day 18 embryonic Mouse brain. These cells are prepared fresh each week and shipped in a nutrient rich medium that keeps the cells alive for up to 14 days under refrigeration.

Shipping/Storage: Primary Mouse Hippocampal Cells are shipped refrigerated. Cells are stable for up to 2 weeks when stored at 4-8ºC. It is recommended to plate the cells as soon as possible after receiving cells according to Application Notes outlined below.

Application Notes

Materials Needed Not Provide

- Poly-D-lysine (Sigma P6407) for substrate
- Papain (Sigma P4762; or Worthington) for enzymatic dissociation
- Trypan blue to count cells to get proper plating density
- 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.)
- Additional media
    - o Neurobasal (Invitrogen 1103-049)
    - o B27 (Invitrogen 17504-044
    - o Glutamine (Invitrogen 35050-061)

General Protocol to Prepare Astrocyte Cultures

Store tissue at 4°C until ready to use for up to 1 week after receipt.

Substrate Preparation

1. Prepare culture plate by coating with poly-D-lysine (0.15 ml/cm^2, 50 µg/ml, 135 kD) 1-20 hr., and rinse one time with 18 Mohm deionized water, and let dry.

Preparation of Astrocyte Culture

Option 1: Mechanical Dissociation (fast procedure; lower yield)

a. Transfer 1 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.

b. 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.

c. Proceed to Step 1 below

Neuromics • 5325 West 74th Street, Suite 8 • Edina, MN 55439
phone 866-350-1500 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

Option 2: Enzymatic and Mechanical Dissociation (longer procedure; higher yield)

a. 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.

b. 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.

c. Add 2 ml of media made in step 2 (Hibernate-Ca containing 2mgs/ml of papain). Incubate for 30 minutes at 30°C.

d. Remove enzymatic solution, again careful not to disturb or remove tissue, add back 1 ml of media saved in 15 ml tube.

e. 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.

f. Proceed to Step 1 below

1. Let undispersed pieces settle by gravity for 1 min.

2. Transfer the dispersed cells (supernatant) into the 15 ml tube that contains the 1ml of media from and gently mix the cells by swirling.

3. 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.

4. Flick the tube a few times to loosen the cell pellet. Resuspend pellet in 1 ml of the provided Neurobasal/10% Serum/3 mM glutamine medium. Resuspend cells by gently pipetting up and down.

5. Aliquot 20 µl and mix with 20 µl of 0.4% trypan blue.

6. Count cells with a hemocytometer.

7. Further dilute the cells with Neurobasal/10% Serum/3 mM glutamine to the desired plating density. We recommend 15×10^3 cells/2 cm^2 of substrate in 0.4 ml/2 cm^2 substrate. Plating at higher densities will result in a mixture of neurons and astrocytes.

8. Incubate the cells at 37ºC with 5% CO_2 and 9% (20% oxygen is fine as well).

9. After 4-6 days astrocytes will be nearly confluent and ready to harvest or pass. If desired, you can change 50% of the medium to Neurobasal / 2% B27/0.5mM glutamine in preparation to harvest the next day for transfer to neuron cultures. If expansion is desired, harvest cells with 0.05% trypsin in Hibernate-Ca, 37ºC, 5 minutes. Pellet cells as in step 3 and continue with steps 4-9.