NEUROMICS

Primary Mouse Cerebellum

Catalog Number: PC35139

Components: ● **E18 Mouse** Cerebellum
● **Media-** enough to initiate a culture

Description: Fresh E18 intact brain tissue from a C57 mouse and media provided to initiate a culture of cerebellum. This tissue allows you to create a cerebellum culture that retains its physiological functions.

Shipping/Storage: Use tissue immediately for highest cell yield; however, tissue can be stored for one week at 4-8°C.

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
    - Neuromics' NbActiv4-M36107

  OR
    - Neurobasal (Invitrogen 21103-049)
    - B27 (Invitrogen 17504-044)
    - Glutamine (Invitrogen 35050-061

Substrate Preparation

1. Prepare culture plate by coating with poly-D-lysine (0.15 ml/cm2 , 50 µg/ml, 135 kD) 1-20 hr., and rinse one time with 18 Mohm deionized water, and let dry.

Preparation of Isolated Neurons

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. 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.
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.

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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 the provided B27/Neurobasal/0.5 mM glutamine medium. Resuspend cells by gently pipetting up and down. For E18 Hippocampus, media includes 25uM glutamate.
11. Aliquot 20 µl and mix with 20 µl of 0.4% trypan blue.
12. Count cells with a hemocytometer.
13. Further dilute the cells with B27/Neurobasal/0.5 mM glutamine to the desired plating density. We recommend 32 x 103 cells/2 cm2 of substrate in 0.4 ml/2 cm2 substrate.
14. Incubate the cells at 37ºC with 5% CO2 and/or 9% or 20% oxygen.
15. After 4 days or longer, neurons are well differentiated. If further culture is desired, change half of medium with fresh, warm B27/Neurobasal/0.5 mM glutamine, without glutamate. Change half the medium every 3-4 days. Additional media and media supplements will need to be purchased to culture neurons past 4-6 days.

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