NEUROMICS

E18 combined Hippocampus, Cortex, and Ventricular Zone Cells

Catalog Number: PC35103

Components :
- **Approximately 1 x 10^6 cells** (2 mls) E18 Sprague/Dawley or Fischer 344 combined Hippocampus, Cortex, and Ventricular Zone neurons.
- **12 mls Culture Media-** Neurobasal/B27/0.5 mM glutamine/25 µM glutamate culture medium, **5 mls Hibernate** Shipping Media

Description: Primary Rat Combined Hippocampus, Cortex, and Ventricular Zone Cells are live neurons isolated from micro-surgically dissected regions of day 18 embryonic Sprague/Dawley or Fischer 344 rat brain. These cells are prepared fresh each week and shipped in a nutrient rich media that keeps the cells alive for up to 7 days under refrigeration. The cells are ideal for a wide variety of applications including: transfection, pharmacology, electrophysiology, immunocytochemistry, and neuronal development studies.

Shipping/Storage: Primary Rat Combined Hippocampus, Cortex, and Ventricular Zone Cells Cells are shipped refrigerated. Cells are stable for up to 7 days 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 (Worthington or Sigma P4762) 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
- NbActiv4 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/cm^2, 50 µg/ml, 135 kD) 1-20 hr., and rinse one time with 18 Mohm deionized water, and let dry.

Preparation of Isolated Neurons

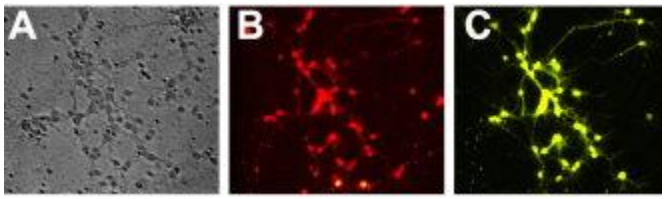
1. Store tissue at 4°C until ready to use.
2. When ready to plate, make up 2 mls of enzymatic solution in shipping media without B27 (Hibernate-Ca; 5 mls supplied) containing 4 mgs (2mgs/ml) of papain. Make sure to sterile filter solution with a 0.2 micron filter after adding papain if source of enzyme 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. 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 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 10^3 cells/2 cm^2 of substrate in 0.4 ml/2 cm^2 substrate.
14. Incubate the cells at 37ºC with 5% CO_2 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.

Viability Assay

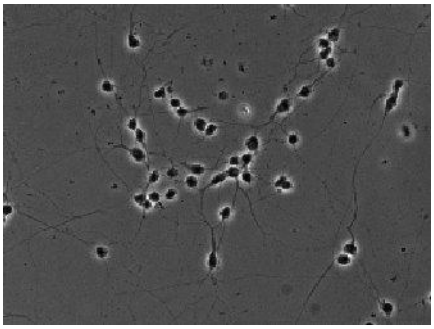
1. Rinse cells twice with PBS.
2. From an acetone stock of 15 mg/ml fluorescein diacetate (Sigma), add 15 µl (1:100 dilution of the stock) into 1.5 ml HBSS. From an aqueous stock of 4.6 ml/ml propidium iodide, add 15 µl of the stock into the same 1.5 ml HBSS (1:100 dilution). Add 40 µl of that dilution to each well with 0.4 ml HBSS (further 1:100 dilution).
3. After approximately 1 minute, count using Nikon B1A filter or other blue excitation appropriate for fluorescein fluorescence. Green cells are alive. Small red nuclear stain indicates a dead cell.
4. If desired, fix and stain with 0.25% Coomassie blue R in ethanol/HAc/water (45/10/45), 1 min., rinse with 10% HAc, aspirate and dry.

Customer Data



Primary rat neurons: Transfection of functional HeV glycoproteins and infection with HeV pseudotyped virions.In order to establish the feasibility of carrying out the proposed experiments in primary neurons, we show that our assays are amenable to use in primary neurons. In the experiment, Combined Hippocampus, Cortex, and Ventricular -E18 (Neuromics) were plated, and at 3 days were transfected with plasmids encoding HeV G/F as well as YFP. On the following day, these cells were infected with HeV or VSV pseudotyped viruses bearing RFP. In the figure, (A) the phase contrast photos show the differentiated neurons; (B) upon excitation for RFP, the red fluorescence indicates neurons infected by HeV pseudotyped virions; (C) upon excitation for YFP, and the green fluorescence shows the efficiency of transfection in neurons. This experiment indicates that the proposed experiments can be carried out in primary neurons, which are transfectable and infectable in our systems, and thus supports all the proposed aims. *Data Courtesy of Dr. Matteo Porotto, Weill Cornell Medical College.* Larger Image

Combined Hippocampus, Cortex, and Ventricular 20X Image Courtesy of Dr. Lidia Garner, University of Tennessee HSC. Link to Larger Image.



"I got 10 million cells total after extraction from the tissue. At Day 4 they all developed long axons. Thank you so much for the replacement**."**