NEUROMICS

E18 Primary Rat Cerebellar Cells

Catalog Number: PC35103 **Components :**
- **Approximately 1 x 10^7 cells** (2 mls) E18 Sprague/Dawley Cerebellar neurons.
- **12 mls Culture Media-** Neurobasal/B27/0.5 mM glutamine/25 µM glutamate culture medium

Description: Primary Rat Cerebellar Cells are live neurons isolated from micro-surgically dissected regions of day 18 embryonic Sprague/Dawley rat brain. These cells are prepared fresh each week and shipped in a nutrient rich medium that keeps the cells alive for up to 7 days under refrigeration.

Shipping/Storage: Primary Rat Cerebellar Cells are shipped refrigerated. Cells are stable for up to 1 week 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
  - Neuromics' NbActiv1-M36109

  OR
  - Neurobasal/B27 **without glutamate** Glutamine (Invitrogen 35050-061)

Preparation (Room Temperature in a Sterile Hood

1. Prepare substrate by coating with 50 µg/ml poly D-lysine (0.15 ml/cm^2) (Sigma P63407). Incubate coated surfaces for at least 1 hour (up to 20). Aspirate the poly-D-lysine, rinse once with ddH_2O, aspirate and air dry.
2. Prepare cell dissociation solution by dissolving 6 mg sterile papain in 3 ml of Hibernate E-Ca (HE-Ca) without B27 (Neuromics M36101-5 supplied) for a final working concentration of 2 mg/ml. Incubate for 10 minutes at 30°C to dissolve.
3. Fire polish the tip of a sterile 9" silanized Pasteur pipette to an opening of ~0.5 mm
4. Aliquot 80 µl of Trypan Blue (Sigma T8154) into a 0.5 mL tube for Step 9.

Cell Dispersal (Room Temperature in a Sterile Hood)

1. With the silanized Pasteur pipette, carefully transfer HEB solution to a sterile tube (save for Step 3) leaving tissue with minimal HEB
2. Add 2 ml of cell dissociation solution to the tissue and incubate for 10 minutes at 30°C. Gently swirl every 5 minutes
3. Remove cell dissociation solution leaving the tissue at the bottom. Return HEB from Step 1
4. With the silanized Pasteur pipette, triturate tissue for about 1 minute (90% tissue dispersal) avoiding air bubbles
5. Let undispersed pieces settle for 1 minute
6. Transfer supernatant containing dispersed cells to a sterile 15 ml tube. Leave ~50 µl of HEB containing debris
7. Spin 1100 rmp (200 x G), 1 minute. Discard supernatant leaving ~50 µl of HEB containing the pellet.

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

8. Disperse the pellet of cells (flick the bottom of the tube with a finger) and resuspend pellet in 1 ml NbActiv1™
9. Aliquot 20 µl of cell solution into the 0.5 ml tube containing 80 µl of Trypan Blue (1:5 dilution)
10. Count cells using a hemacytometer (calculate cells/ml)

Cell Plating (Room Temperature in a Sterile Hood)

1. Dilute cells with NbActiv1™ (0.2 ml/cm^2) and plate at 16,000 cells/cm^2 or desired concentration.
2. Incubate 37°C, 5% CO_2, 9% O_2, 95% humidity (or ambient O_2)
3. After 4 days, neurons display axons and dendrites; synapses and action potentials begin at 7 days.
4. Change ½ of the medium with fresh, 37°C, CO_2 equilibrated NbActiv1™ every 3-4 days.
    a. 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.