NEUROMICS

E18 Primary Rat Striatum Cells

Catalog Number: PC35105 **Components :** ●**Striata Pair** (2 mls) E18 Sprague/Dawley neurons.
●**12 mls Culture Media-** Neurobasal/B27/0.5 mM glutamine/25 µM glutamate culture medium

Description: Primary Rat Striatum 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. The cells are ideal for a wide variety of applications including: transfection, pharmacology, electrophysiology, immunocytochemistry and neuronal development studies.

Shipping/Storage: Primary Rat Striatum 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.

References:

Shouqing Luo, Haruo Mizuta and David C. Rubinsztein. p21-activated kinase 1 promotes soluble mutant huntingtin selfinteraction and enhances toxicity. Human Molecular Genetics 2008 17(6):895-905; doi:10.1093/hmg/ddm362 ...Rat E18 cortical and striatal neurons were purchased from Neuromics (MN) or prepared from E18 rat brain tissues according to instructions from Neuromics, cultured in neurobasal (Invitrogen) medium supplemented with B27 (Invitrogen) and 0.5 mM glutamine and transfected with Lipofectamine 2000 according to standard methods...

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.
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.

Image: *Striatal Neuron Culture. Courtesy of Dr. Henry Guo, Intracellular Therapies, Inc*