



E18 Primary Rat Substantia Nigra Cells

Catalog Number: PC35125

Components: • **Approximately 5 x 10⁶ cells** (2 mls) E18 Sprague Dawley

Substantia Nigra neurons.

• **12 mls Culture Media-** Neurobasal/B27/0.5 mM glutamine/25 μ M glutamate culture medium, **5 mls Hibernate Shipping Media**

Description: Primary Rat Substantia Nigra 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 Substantia Nigra Cells are shipped refrigerated. Cells are stable for up to 7 days when stored at 4-8°C. 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 Provided

- 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' [NbActiv1-M36109](#)
 - OR
 - Neurobasal/B27 **without glutamate**
 - Glutamine (Invitrogen 35050-061)

Primary Neuron Culture Protocol

Preparation (Room Temperature in a Sterile Hood)

1. Prepare substrate by coating with 50 μ g/ml poly D-lysine (0.15 ml/cm²) (Sigma P63407). Incubate coated surfaces for at least 1 hour (up to 20). Aspirate the poly-D-lysine, rinse once with ddH₂O, 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 μ l 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

FOR RESEARCH USE ONLY V5-06/2011

Neuromics' reagents are for in vitro and certain non-human in vivo experimental use only and not intended for use in any human clinical investigation, diagnosis, prognosis, or treatment. We disclaim all liability in connection with the use of the information contained herein or otherwise, and all such risks are assumed by the user.

5. Let undispersed pieces settle for 1 minute
6. Transfer supernatant containing dispersed cells to a sterile 15 ml tube. Leave ~50 μ l of HEB containing debris
7. Spin 1100 rpm (200 x G), 1 minute. Discard supernatant leaving ~50 μ l 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 μ l of cell solution into the 0.5 ml tube containing 80 μ l 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/cm²) and plate at 16,000 cells/cm² or desired concentration.
2. Incubate 37°C, 5% CO₂, 9% O₂, 95% humidity (or ambient O₂)
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, CO₂ 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 μ l (1:100 dilution of the stock) into 1.5 ml HBSS. From an aqueous stock of 4.6 ml/ml propidium iodide, add 15 μ l of the stock into the same 1.5 ml HBSS (1:100 dilution). Add 40 μ l 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.

FOR RESEARCH USE ONLY V5-06/2011

Neuromics' reagents are for in vitro and certain non-human in vivo experimental use only and not intended for use in any human clinical investigation, diagnosis, prognosis, or treatment. We disclaim all liability in connection with the use of the information contained herein or otherwise, and all such risks are assumed by the user.

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