



## E18 Primary Rat Cortical Astroglia Cells

Catalog Number: PC36108

Components : •1 Cortical Pair (2 mls) -Approximately  $1 \times 10^6$  cells/Cortical Pair  
•12 mls Astroglial Culture Media-; **NbAstro™**; 5 mls Hibernate-Ca without B27

**Description:** E18 Primary Rat Cortical Astrocytes are live cells provided as 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 media (Hibernate-Ca) that keeps them alive for up to 7 days under refrigeration. Following simple dissociation steps, the cells can be quickly plated on almost any poly-lysine coated substrate using the provided Astroglial Culture Media (12 ml **NbAstro**.) The cells are ideal for a wide variety of applications including: transfection, pharmacology, electrophysiology, immunocytochemistry, and neuronal development studies.

**Shipping/Storage:** Primary Rat Cortical 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 Provide

- Poly-D-lysine (Sigma P6407) for substrate
- Papain (Sigma P4762; or Worthington) for enzymatic dissociation
- 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-for transfer to neuron cultures:
  - **NbActiv1™** (cat#: M36109-100) or Neurobasal®/B27®/GlutaMAX™

#### Substrate Preparation (room temperature in a sterile hood)

1. Prepare substrate by coating with 50 µg/ml poly-D-lysine (0.15 ml/cm<sup>2</sup>) (Sigma P6407). Incubate coated surfaces for at least 1 hour (up to 20). Aspirate the poly-D-lysine, rinse once with ddH<sub>2</sub>O, 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 for a final working concentration of 2 mg/ml papain. 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 the tissue with minimal HEB.
2. Add 2 ml of cell dissociation solution to the tissue and incubate for 10 min at 30°C. Gently swirl every 5 min.
3. Remove cell dissociation solution leaving the tissue at the bottom. Return the HEB medium from step 1.
4. With the silanized pasteur pipette, triturate tissue for about 1 min (90% tissue dispersal) avoiding air bubbles.
5. Let undispersed pieces settle for 1 min.
6. Transfer supernatant containing dispersed cells to a sterile 15 ml tube. Leave ~50 µl of HEB containing debris.
7. Spin 1100 rpm (200 x G), 1 min. 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 of Astroglial medium (NbASTRO).
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).

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**Cell Plating (room temperature in a sterile hood)**

1. Dilute cells with NbASTRO (0.2 ml/cm<sup>2</sup>) and plate at 7,500 cells/cm<sup>2</sup> or desired concentration. Note: Plating at higher densities will result in a mixture of neurons and astrocytes.
2. Incubate 37°C, 5% CO<sub>2</sub>, 9% O<sub>2</sub>, 95% humidity (or ambient O<sub>2</sub>).
3. After 4-6 days, astrocytes will be 90% confluent and ready to harvest or pass.
4. Transfer to neuron cultures: 24 hours prior, change ½ of the medium to [NbActiv1 \(cat#: M36109-100\)](#) or Neurobasal@/B27@/GlutaMAX™
5. For expansion: Harvest cells with 0.05% trypsin in HE-Ca, (37°C, 5 min). Pellet cells as in step 7 and continue with steps 8-10 in Cell Dispersal and Steps 1 & 2 in Cell Plating.

**Viability Assay**

1. Rinse twice with 37°C HBSS (0.2 ml/cm<sup>2</sup> of substrate).
2. Prepare dye mix from an acetone stock of 15 mg/ml fluorescein diacetate and an aqueous stock of 4.6 µg/ml propidium iodide, dilute 15 µl of each into 1.5 ml HBSS (1:100 dilution).
3. Add 20 µl of dye mix from step 2 to every 0.2 ml of HBSS added in step 1 (1:10 dilution).
4. After ~1 min count live cells using blue excitation appropriate for fluorescein fluorescence (green cells). Count dead cells with green excitation for propidium iodide fluorescence (small red nuclei).
5. Viability = (green cells/unit area)/(total cells plated/unit area) or Survival = green cells/(green + red cells).

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