NEUROMICS

E18 Primary Mouse Cortical Astroglia

Catalog Number: PC36111

Components :
- 4 Cortical Pair (in 2 mls of Hibernate® EB (HEB; Hibernate E®/B27®/GlutaMAX™) -Approximately 1 x 10^6 cells/Cortical 4 Pair
- 12 mls Astroglial Culture Media-; NbAstro™; 5 mls Hibernate® EB (HEB; Hibernate E®/B27®/GlutaMAX™)

Description: C57 Primary Mouse Cortical Cells are live neurons isolated from micro-surgically dissected regions of day 18 embryonic Mouse brain. These cells are prepared fresh each week and shipped in a nutrient rich medium

Shipping/Storage: Primary Rat Cortical 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 (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™

SubstrPreparations (Room Temperature in a Sterile Hood)

1. Prepare substrate by coating with 50 µg/ml poly-D-lysine (0.15 ml/cm2) (Sigma P6407). 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 EB (5 mls provided) for a final working concentration of 2 mg/ml papain. Incubate for 10 minutes at 30ºC 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.ate Preparation (room temperature in a sterile hood)

Cell Dispersal (room temperature in a sterile hood)

1. With the silanized pasteur pipette, carefully transfer tissue storage 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^2) and plate at 7,500 cells/cm_2 or desired concentration. Note: Plating at higher densities will result in a mixture of neurons and astrocytes.
2. Incubate 37°C, 5% CO_2, 9% O_2, 95% humidity (or ambient O_2).
3. After 4-6 days, astrocytes will by 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 37ºC HBSS (0.2 ml/cm^2 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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