NEUROMICS

E18 Primary Dorsal Root Ganglia (DRGs)

Catalog#: PC35115

Components Included: 10 ganglion from one, embryonic day 1 frpm Sprague Dawley rat.
Storage Media-2 ml Hibernate A®/B27®/GlutaMAX™=HAB
Dissociation Media-5 ml Hibernate A without Calcium and Magnesium=HA -Ca -Mg **Culture and Growth Media-**12 ml NbActiv4 + NGF included.

Description: Primary Rat DRGs are live neurons isolated from micro-surgically dissected regions of day 18 embryonic Sprague/Dawley rat. These cells are prepared fresh each week and shipped in a nutrient rich medium that keeps the cells alive for up to 7days under refrigeration. The cells are ideal for a wide variety of applications including: transfection, pharmacology, electrophysiology, immunocytochemistry, and neuronal development studies

Shipping/Storage: DRGs 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.

Protocol

Additional Components Required:

Dissociation Enzyme: Collagenase/Dispase (Roche 10 269 638 001).
Coating: Poly-D-Lysine (Sigma P6407).
Additional Growth Media: NbActiv4 + NGF Please contact Brett @ 866-350-1500 or brett@neuromics.com for additional media You will need to add NGF (Invitrogen 13290-010) to the NbAvtiv4 (Catalog #: M36107).
Coverslips and dishes: 15mm round glass coverslips (Carolina Biological Supply #63-3031) and sterile 35mm perti dish (VWR# 25382-064).

Preparations (Room Temperature in a Sterile Hood):

1. Prepare substrate by coating with poly-D-lysine (100 µg/ml). 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 a 100 mg/ml stock solution of Collagenase/Dispase (Roche; Ref: 10 269 638 001) in ddH_2O.
3. To make the cell dissociation solution, dilute Step 2 to 1:100 with Hibernate A without Calcium and Magnesium (HA Ca -Mg) for a final working solution of 1 mg/ml collagenase (0.1 U) / dispase (0.8 U). *Solution must be sterile filtered prior to use*. It can be aliquoted and frozen @ -20ºC and thawed day of use.
4. Fire polish the tip of a sterile 9" silanized pasteur pipette to an opening of ~0.5 mm
5. Aliquot 20 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 remove the storage solution leaving the DRG's in minimal solution.
2. Place tissue in cell dissociation solution (Step 3, above) for 1 hr and incubate at 37ºC (ambient atmosphere). Gently swirl every 5 min.
3. Allow tissue to settle and remove cell dissociation solution leaving the tissue at the bottom in minimal solution.
4. Immediately add 2 ml of Hibernate A®/B27®/GlutaMAX™ (HAB).
5. With the silanized pasteur pipette, triturate tissue ~30 times until 90% dispered while avoiding air bubbles.
6. Let undispersed pieces settle for 1 min.
7. Transfer supernatant containing dispersed cells to a sterile 15 ml tube. Leave ~50 µl of HAB containing debris.

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

8. Spin 1100 rpm (200 x G), 3 min. Discard supernatant leaving ~50 µl of HAB containing the pellet.
9. Disperse the pellet of cells (flick the bottom of the tube with a finger) and resuspend pellet in 1 ml NbActiv1® with 25 ng/mL NGF.
10. Aliquot 20 µl of cell solution into the 0.5 ml tube containing 20 µl of Trypan Blue (1:2 dilution).
11. Count cells using a hemacytometer (calculate cells/ml).

Cell Plating (Room Temperature in a Sterile Hood):

1. Dilute cells with NbActiv4® + 25 ng/ml NGF (0.2 ml/cm^2) and plate at 100,000cells/cm^2 or desired concentration.
2. Incubate 37ºC, 5% CO_2, 9% O_2, 95% humidity (or ambient O_2).
3. After 4 days, DRG's display axons and dendrites; synapses and action potentials begin at 7 days.
4. Change ½ of the medium with fresh, 37oC, CO2 equilibrated NbActiv4® + 25 ng/ml NGF every 3-4 days.

Viability Assay:

- Rinse twice with 37ºC HBSS (0.2 ml/cm^2 of substrate).
- Prepare dye mix from an acetone stock of 15 mg/ml fluorescein diacetate and an aqueous stock of 4.6 mg/ml propidium iodide, dilute 15 µl of each into 1.5 ml HBSS (1:100 dilution).
- Add 20 µl of dye mix from step 2 to every 0.2 ml of HBSS added in step 1 (1:10 dilution).
- 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).
- Viability = (green cells/unit area)/(total cells plated/unit area) or Survival = green cells/(green + red cells)
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References

A dissection and Tissue Culture Manual of the Nervous System (1989). A. Shahar, J.D. Vellis, A. Vernadakis, B. Haber (Eds.), *Dissociated Spinal Cord - Dorsal Root Ganglion Cultures on Plastic Tissue Culture Dishes and Glass Coverslips and Wells* (pp.219-222). Wiley-Liss, Inc.

J.L. Werth, S.A. Thayer (1994) Mitrochondria Buffer Physiological Calcium Loads in Cultured Rat Dorsal Root Ganglion Neurons, *The Journal of Neuroscience,* 14(1), 348-356.

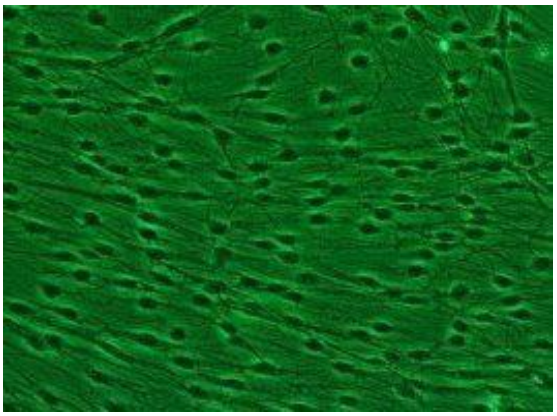

Image: DRGs on Poly-D-Lysine

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