NEUROMICS

Cortical Neurons Kit

Catalog #: PC35117

Product Format: T25 Flask

Enclosed is a T25 culture flask containing live adherent primary cortical neurons obtained from Sprague Dawley rats and a 12 ml tube of NbActiv4® culture medium (Neurobasal®/B27®/GlutaMAX™). Live adherent neurons are stored in ~65ml of our shipping medium. For cell survival, an immediate media exchange is required.

Preparations:

1. Remove the T25 culture flask and 12 ml tube of NbActiv4® medium from the shipping container
2. Allow both the T25 culture flask and the 12 ml tube of NbActiv4® medium to equilibrate to room temperature

Media Exchange (Room Temperature in a Sterile Hood)

1. Remove the parafilm seal
2. Sterilize the T25 culture flask lid with 70% EtOH and allow to air dry
3. For each T25 culture flask (one flask at a time)
4. Aspirate off the shipping media
5. Immediately add 5.0 mL of NbActiv4® medium to the T25 culture flask
6. Repeat for all remaining T25 flasks
7. Incubate the T25 culture flask(s) at 37°C, 5% CO_2, 9% O_2, 95% humidity (or ambient O_2)
8. Upon receipt, neurons display axons and dendrites; synapses and action potentials begin at 7 days
9. Change ½ of the medium with fresh, 37°C, CO_2 equilibrated NbActiv4® every 3-4 days.

Viability Assay:

1. Rinse twice with 37°C 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 mg/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 diultion)
4. Afer ~1 minute count live cells using blue excitation appropriate for fluorescein fluorescence (green cells). Count dead cells with green excitation for propidium iodide fluoscence (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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