NEUROMICS

# Cortical Astroglia Kit

**Catalog #:** PC35131

**Product Format:** T25 Flask

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Enclosed is a T25 culture flask containing live adherent primary cortical astroglia obtained from E18 Sprague Dawley rats and a 12 ml tube of NbASTRO culture medium (Neurobasal®/Horse Serum/GlutaMAX™). Live adherent astroglia 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 NbASTRO medium from the shipping container
2. Allow both the T25 culture flask and the 12 ml tube of NbASTRO 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)
    a. Aspirate off the shipping media
    b. Immediately add 5.0 mL of NbASTRO medium to the T25 culture flask
    c. Repeat for all remaining T25 flasks
4. Incubate the T25 culture flask(s) at 37°C, 5% $CO_2$, 9% $O_2$, 95% humidity (or ambient $O_2$)
5. Change ½ of the medium with fresh, 37°C, $CO_2$ equilibrated NbASTRO every 3-4 days.
6. After 12-14 days, astroglia will be 90% confluent and ready to harvest or passage.

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. After ~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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