Neuro-2a cell culture (Neuro 2a, N2a)

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Abstract

Basic Protocol for culturing Neuro2a cells.

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Guidelines

The following are taken directly from the vendor website, I use high glucose DMEM instead of Eagle's MEM.

Complete Growth Medium The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Subculturing Volumes are given for a 75 cm2 flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

Materials

DMEM, High Glucose 11965-092 by Life Technologies

Fetal Bovine Serum, qualified 10437-028 by Life Technologies

Neuro-2a (ATCC® CCL-131™) CCL-131 by ATCC

Protocol

Step 1.
For a T-75 flask:

Remove and discard culture medium.

Step 2.
Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.

Step 3.
Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

Step 4.
Add 10 mL of complete growth medium and aspirate cells by gently pipetting.

Step 5.
Pipett 10mL cell suspension through 60um filter into 40mL to achieve a 1:5 split

Step 6.
Plate cells in appropriate vessel (T-75 flask or 6-well plate) and incubate at 37C

Step 7.
Change media every 2 - 4 days

Step 8.
Resubculture when cells are 70 - 90% confluent