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Adult Mouse Glial Cell Line Maintenance

Designation:

Adult Mouse Astrocyte Cell Line mHypoA-Ast1 (male; Hypothalamus derived)
Adult Mouse Astrocyte Cell Line mHypoA-Ast1/2 (male; Hypothalamus derived)
Adult Mouse Glial Cell Line mCortA-G1 (mixed; female; Cortex derived)
Adult Mouse Oligodendrocyte Cell Line mBstA-Olig1 (female; Brainstem derived)

Catalogue Numbers: CLU529 – CLU532

Description:

This product line is based on proprietary platform technology which has enabled the creation of a series of immortalized glial cell lines isolated from mouse brain. These neurons were immortalized via retroviral transfer of SV40 T-Ag into glial cell primary cultures isolated from various regions (hypothalamus, cortex and brainstem) of adult CD-1 mice brains. These cell lines have been found to express an ever-expanding array of glial cell specific markers. As such, these cell lines enable accurate in-vitro assays for use in the discovery, development and validation of new therapeutics targeted to central-nervous system diseases and disorders, including obesity and neurodegenerative diseases.

Cell culture conditions:

Note: The adult cells typically grow slower when initially thawed than the embryonic mouse cell lines. The adult cells appear to behave more like primary cultures and appropriate attention should be taken to ensure the successful expansion of the cells.

Note: All cell culture should be conducted in a cell culture hood, with sterile conditions. Standard tissue culture materials (plates, pipettes etc.) are suitable.

The cells are grown in DMEM with 10% fetal bovine serum (FBS), 25 mM glucose and 1% penicillin/streptomycin and maintained at 37°C with 5% CO_2 (see below). The cells will grow in a monolayer culture, attached to the tissue culture plate. The cells can be split when they are 70-90% confluent, with a plate ratio of 1:4. Trypsinization is recommended to obtain single cells in suspension (Wash with 1x phosphate buffered saline (PBS), then add 1x trypsin-EDTA (0.5-1 ml per 100 mm plate) at 37°C for 1-5 min, followed by washing/resuspension in growth medium). Be fairly gentle at this stage to avoid cell death. It will usually take 2 days for the plate to become confluent again, if the procedure is followed correctly. These numbers may vary slightly from lab to lab, depending upon technique.

Recommend media requirements:

DMEM: Sigma D5796 (with 4500 mg/L glucose, L-glutamine (0.584 g/L), sodium bicarbonate (3.7 g/L) without sodium pyruvate)
Fetal Bovine Serum (US): ATCC 30-2020
Penicillin/Streptomycin, Liquid: Sigma, P4333, contains 10,000 units of penicillin and 10 mg of streptomycin/ml
Trypsin-EDTA: Invitrogen 15400-054

Cell line thawing:

The cell vial is removed from liquid nitrogen and thawed quickly in a 37°C water bath. The cells are initially incubated in a 60 mm tissue culture plate in growth medium (DO NOT centrifuge), as described above.

IMPORTANT NOTE: The same day, after the cells have attached to the plate (approximately 4-6 h), the medium should be refreshed to remove the DMSO. (If this procedure is not followed and the DMSO is removed the following day, the cells will likely be dead.)

Cell line freezing:

It is highly recommended to freeze a few aliquots of the cells immediately after the initial growth/split to avoid losing the cell line. Freezing medium is the same as the growth medium described above, but supplemented with 10% sterile dimethylsulfoxide (DMSO).
Target concentration of cells is 10^5/ml of freezing medium. Cryogenic vials are placed in a NALGENE™ Cryo 1°C Freezing Container overnight in a -80°C freezer. The next day the vials are transferred to a liquid nitrogen tank. It is recommended to test the cells for regrowth after freezing to be sure that the freezing procedure was performed correctly.

References:

Loganathan N, Lieu CV, Belsham DD. (2024) **Immortalization and Characterization of GFAP-expressing Glial Cells from the Adult Mouse Hypothalamus, Cortex, and Brain Stem**. Neuroscience. 551:43-54.