Adult Mouse Hypothalamic SCN mix Cell Line Maintenance

Designation: mHypoA-SCN mix
Catalogue Numbers: CLU497

Description:

This product is based on a proprietary platform technology which has enabled the creation of a series of immortalized hypothalamic neurons. Primary neurons were micro dissected from the suprachiasmatic nucleus (SCN) area of the hypothalamus from 6 adult female C57Bl/6 mice. Pooled primary cultures were immortalized by retroviral transfer of the SV40 T-Ag. No further sub-cloning of the immortalized cells was done.

This cell line has been found to express an ever expanding array of neuropeptides, enzymatic markers and biologically active receptors. As such, this cell line will 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, stress, and metabolic disorders, amongst others.

Cell culture conditions:

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

The cells are grown in 1x 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.

Recommended 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: Biochrome AG, A2213, contains 10,000 units of penicillin and 10,000 μg 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, 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.