Mouse Motor Neuron (NSC-34) Cell Line Maintenance

Catalogue #: CLU140

Description:

NSC-34 is a hybrid cell line, produced by fusion of motor neuron enriched, embryonic mouse spinal cord cells with mouse neuroblastoma. Cultures contain two populations of cells: small, undifferentiated cells that have the capacity to undergo cell division and larger, multi-nucleate cells. These cells express many properties of motor neurons, including choline acetyltransferase, acetylcholine synthesis, storage and release and neurofilament triplet proteins.

Applications: NSC-34 cells have been evaluated following exposure of cultures to a selection of chemicals know to be neurotoxic to motor neurons. NSC-34 cells respond to agents that affect voltage-gated ion channels, cytoskeletal organization and axonal transport. The sensitivity of action potential production to various ion channel blockers is similar to that in primary motor neurons in culture. Therefore, these immortalized motor neuron-like cells have the utility as a model for the investigation of neurotoxicity.

Cell culture conditions:

Media:
The cells grow in a high glucose formulation of DMEM supplemented with 10% FBS. Although not an absolute requirement, the cells can tolerate Pen/Strep used at 1X. Glutamine can also be added at 2-7mM.

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: Biochrome AG, A2213, contains 10,000 units of penicillin and 10,000 μg of streptomycin/ml

Thawing:
The cryovial 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. The cells can be diluted 5-10 fold for the initial plating. It is not recommended to centrifuge the cells before plating, although it is known to be done. It has been found that the DMSO is less of a problem to the cells immediately upon thawing.

***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.)

Culture:
The cells grow right on the surface of the conventional tissue culture plastic; no special coating in required. Cell density is not a concern for the first few days after thawing because it is viewed more as a recovery period. Once they’re growing properly then they can be plated at whatever density is most appropriate for the experiment being done.

Medium is typically changed every 2-3 days, depending on rate of growth. Cultures should be split at ~80% confluence 1:3-1:4. Remove cells from plate/flask using 0.25% trypsin-EDTA solution (~2 ml for 60-100 mm plate or T25 flask, 3 min at 37°C) and dilute 10X right away with DMEM.
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.

The freezing medium recommended is as follows: 60% growth medium described above, 30% FBS supplemented with 10% sterile dimethylsulfoxide (DMSO) however variations of freezing media are acceptable. Target concentration of cells is 10^7/ml of freezing medium. Typically a confluent 100 mm plate or 50 ml culture flask will yield 6-10 cryovials.

The cells can be removed from the plate by trituration or using trypsin (as described above). The cells are centrifuged and the supernatant is removed. The cell pellet is then re-suspended in the small amount of liquid retained by surface tension. If this step is missed, the cells may clump when the larger volume of freezing medium is added. After re-suspending, the appropriate amount of freezing medium is added (6-10 mls) and the suspension is aliquoted into Cryogenic vials (~ 1ml/vial). The vials are then placed in a NALGENETM 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:


