Human Oligodendrocytic (Glial) (MO3.13) Cell Line Maintenance

This is an immortal human-human hybrid cell line that express phenotypic characteristics of primary oligodendrocytes, and was created by fusing a 6-thioguanine-resistant mutant of the human rhabdomyosarcoma RD (cancer of skeletal muscle) with adult human oligodendrocytes by a lectin-enhanced polyethylene glycol procedure. In contrast to the tumor parent, MO3.13 expressed surface immunoreactivity for galactosyl cerebroside (GS) and intracellular immunoreactivity for myelin basic protein (MBP), proteolipid protein (PLP), and glial fibrillary acidic protein (GFAP). Other articles have reported that the MO3.13 also exhibits the markers of immature oligodendrocytes GalC (galactosylceramidase) and CNPase. Upon differentiation, the MO3.13 cells have been also shown to express the mature oligodendrocyte markers MBP and MOG (myelin oligodendrocyte glycoprotein).

Cell culture conditions:

Media:
The cells grow in the high glucose formulation of DMEM supplemented with 10% FBS. Although not an absolute requirement, the cells can tolerate Pep/Strep used at 1X.

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, Qualified (US): PAA A15-751
Penicillin/Streptomycin, Liquid: Biochrome AG, A2213, contains 10,000 units of penicillin and 10,000 μg of streptomyein/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 than the sensitivity of 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-10.

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 10^5/ml of freezing medium. Cryogenic vials are 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.

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