

Data Sheet

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BCRJ Code: 0191

Cell Line: NIH/3T3

Species: Mus musculus

Vulgar Name: Mouse

Tissue: Embryo

Cell Type: Fibroblast

Morphology: Fibroblast

Disease: Normal

Growth Properties: Adherent

Age/Ethnicity: embryo /

Applications: This cell line is a suitable transfection host. The NIH/3T3 cell line is highly sensitive to sarcoma virus focus formation and leukemia virus propagation and has proven to be very useful in DNA transfection studies. [PubMed: 222457]

Virus Susceptibility:: Murine leukemia virus , Murine leukemia virus

Biosafety: 1

Culture Medium: Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 2 mM L-glutamine, 4500 mg/L glucose and 10% of bovine calf serum.

Subculturing:

DO NOT ALLOW THE CELLS TO BECOME CONFLUENT Subculture at least twice per week at 80% confluence or less. Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum which contains trypsin inhibitor. 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 to 15 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. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C. NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010.

**Subculturing
Medium Renewal:**

Twice per week

**Subculturing
Subcultivation Ratio:**

Inoculate 3 to 5 X 10³ cells/cm².

Culture Conditions:

Atmosphere: air, 95%; carbon dioxide (CO₂), 5% Temperature: 37°C

Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:

SAFETY PRECAUTION: It is highly recommended that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the vial and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. For cells that are sensitive to DMSO it is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g for 5 to 7 minutes.
4. Discard the supernatant and Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio).
5. Incubate the culture in an appropriate atmosphere and temperature (see "Culture Conditions" for this cell line).

NOTE: It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).

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The NIH/3T3, a continuous cell line of highly contact-inhibited cells was established from NIH Swiss mouse embryo cultures in the same manner as the original random bred 3T3 (ATCC CCL-92) and the inbred BALB/c 3T3 (ATCC CCL-163). The established NIH/3T3 line was subjected to more than 5 serial cycles of subcloning in order to develop a subclone with morphologic characteristics best suited for transformation assays.

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ATCC: CRL-1658