

Banco de Células do Rio de Janeiro

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BCRJ Code: 0019

Cell Line: 3T3-L1

Species: Mus musculus

Vulgar Name: Mouse

Tissue: Embryo

Cell Type: Fibroblast

Morphology: Fibroblast

Growth Properties: Adherent

L1 is a continuous substrain of 3T3 (Swiss albino) developed through clonal **Derivation:**

isolation.

Applications: This cell line is a suitable transfection host.

Products: triglycerides

Biosafety: 1

The cells undergo a pre-adipose to adipose like conversion as they progress **Addtional Info:** from a rapidly dividing to a confluent and contact inhibited state. A high serum

content in the medium enhances fat accumulation [PubMed ID: 4426090].

Dulbecco's Modified Eagle's Medium (DMEM) modified with 4500 mg/L glucose **Culture Medium:**

and bovine calf serum to a final concentration of 10%.



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Subculturing:

NOTE: Never allow culture to become completely confluent. Remove medium, and rinse with PBS without calcium and magnesium. Remove the solution and add an additional 1 to 2 mL of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37°C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. The recommended inoculum is 2 to 3 X 103 cells/cm2. Subculture before cultures become 70 to 80% confluent or when cells reach 5 to 6 X10(4) viable cells/cm2. 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:

2 to 3 times a week

Culture Conditions:

Atmosphere: air, 95%; carbon dioxide (CO2), 5% Temperature: 37°C Growth Conditions: The serum used is important in culturing this line. Calf serum is recommended and not fetal bovine serum.

Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)









Thawing Frozen Cells:

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SAFETY PRECAUTION: It is strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris.

- 1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
- 2. Remove the vial from the water bath as soon as its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions.
- 3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately $125 \times g$ for 5 to 7 minutes.
- 4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio).
- 5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line).

NOTE: It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at its normal pH (7.0 to 7.6).

References:

Green H, Meuth M. An established pre-adipose cell line and its differentiation in culture. Cell 3: 127-133, 1974. PubMed: 4426090 Green H. Triglyceride-accumulating clonal cell line. US Patent 4,003,789 dated Jan 18 1977 Goodrum FD, et al. Adenovirus early region 4 34-kilodalton protein directs the nuclear localization of the early region 1B 55-kilodalton protein in primate cells. J. Virol. 70: 6323-6335, 1996. PubMed: 8709260 Scherer PE, et al. Identification, sequence, and expression of caveolin-2 defines a caveolin gene family. Proc. Natl. Acad. Sci. USA 93: 131-135, 1996. PubMed: 8552590 Kallen CB, Lazar MA. Antidiabetic thiazolidinediones inhibit leptin (ob) gene expression in 3T3-L1 adipocytes. Proc. Natl. Acad. Sci. USA 93: 5793-5796, 1996. PubMed: 8650171

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Cellosaurus: CVCL 0123



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