



3T3-L1

CL-173™

Description

3T3-L1 is a fibroblast that was isolated from the embryo of a mouse. This cell line can be used to study the basic cellular mechanisms associated with diabetes, obesity, and related disorders.

Organism: *Mus musculus*, mouse

Cell Type: fibroblast

Tissue: Embryo

Age: embryo

Morphology: fibroblast

Growth properties: Adherent

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Patent number:

4,003,789

Technical information: ATCC Product Experience does not have technical information on patent deposits that are not produced or characterized by ATCC. Additional information can be found in the corresponding patent available from the patent holder or with the U.S. and/or international patent office.

Storage Conditions

Product format: Frozen

Storage conditions: Vapor phase of liquid nitrogen

Intended Use

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This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submerged in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

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Temperature: 37°C**Atmosphere:** 95% Air, 5% CO₂

Handling Procedures

Unpacking and storage instructions:

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Complete medium:

The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium (ATCC 30-2002). To make the complete growth medium, add the following components to the base medium: bovine calf serum (ATCC 30-2030) to a final concentration of 10%.

Example media preparation:

- 500 mL Dulbecco's Modified Eagle's Medium
- 56 mL bovine calf serum, iron fortified (do not heat inactivate)

Note: The serum used is important in culturing this line. Calf serum is recommended and not fetal bovine serum.

Handling Procedure:

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends 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 submersed 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 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 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. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 150 to 200 x g for 8 to 12 minutes. Discard supernatant.
4. Resuspend the cell pellet with the recommended complete medium and dispense into a 25 cm² culture flask. Note: For this cell line a T25 flask is recommended because of the number of cells provided. Dilute with the recommended complete culture medium (see the lot information on Certificate of Analysis (COA) for the culture recommended dilution ratio). The recommended seeding density for CL-173 is 2.0 x 10³ to 3.0 x 10³ viable cells/cm². 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 complete 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).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

Subculturing procedure:

Protocol: Never allow culture to become completely confluent.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with D-PBS (ATCC 30-2200) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution (ATCC 2101) 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 37C to facilitate dispersal.

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4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels. 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 X10⁴ viable cells/cm².
6. Incubate cultures at 37°C.

Interval: Every three days

Medium Renewal: 2 to 3 times per week

Reagents for cryopreservation: Complete growth medium supplemented with 5% (v/v) DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: 3T3-L1 (ATCC CL-173)

References

References and other information relating to this material are available at www.atcc.org.

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