

## 3T3-L1 Cells | 400107

### General information

#### Description

3T3-L1 cells are a clonal line of preadipocytes derived from mouse embryonic fibroblasts. These cells have become a widely used in vitro model for studying the process of adipogenesis, including adipogenesis and lipogenesis, which is the differentiation of preadipocytes into adipocytes (fat cells). The name "3T3" refers to the transfer (T) protocol that involved transferring the cells every 3 days, and "L1" signifies the particular clone that was isolated.

Initially, 3T3-L1 cells exhibit a fibroblast-like morphology, but upon induction of 3T3-L1 cell differentiation, 3T3-L1 cells change from a preadipocyte to a mature adipocyte state and accumulate lipid droplets, a hallmark of obesity and metabolic syndrome. The differentiation process from 3T3-L1 preadipocytes to 3T3-L1 adipocytes is triggered by a specific cocktail of inducers, typically including dexamethasone, 3-isobutyl-1-methylxanthine (IBMX), and insulin.

As 3T3-L1 adipocytes adopt the characteristics of mature adipocytes, they begin to express genes that are crucial for adipocyte function, such as those coding for enzymes involved in fatty acid metabolism and hormones like leptin and adiponectin, which play vital roles in regulating appetite, energy balance, and insulin sensitivity. Studying 3T3-L1 cell transformations enhances our understanding of adipogenesis and obesity and fat-related diseases, such as type 2 diabetes, by revealing how lipid accumulation in adipocytes leads to cellular dysfunction and broader metabolic issues.

Moreover, the 3T3-L1 cell line is instrumental in investigating the impact of various substances on adipocyte behavior, such as the effect of pharmacological agents on lipolysis or the anti-inflammatory properties of certain diets that may prevent insulin resistance.

3T3-L1 cells have been extensively used to study the molecular and cellular mechanisms underlying adipocyte differentiation, insulin sensitivity, lipid metabolism, and the effects of various nutritional and pharmacological agents on these processes. Given their ability to differentiate into adipocytes and their ease of culture in vitro, 3T3-L1 cells provide a valuable model system for obesity and diabetes research, as well as for the discovery of new therapeutic targets related to metabolic disease.

**Organism** Mouse

**Tissue** Embryonic

**Metastatic site** Not applicable (embryonic preadipocyte; non-tumorigenic)

**Applications** 3T3-L1 cells have been used as a model system for understanding the molecular mechanisms that regulate adipogenesis and lipid metabolism, and have been utilized in research related to obesity, diabetes, and metabolic diseases. They are also a viable transfection host.

**Synonyms** 3T3 L1, 3T3L1, 3T3-L1 ad, NIH-3T3-L1, NIH3T3-L1

### Characteristics

**Breed/Subspecies** Swiss albino

## 3T3-L1 Cells | 400107

<b>Age</b>	Embryo
<b>Gender</b>	Male
<b>Morphology</b>	Fibroblast-like
<b>Cell type</b>	Preadipocyte / adipocyte (upon differentiation)
<b>Growth properties</b>	Adherent

### Regulatory Data

<b>Citation</b>	3T3-L1 (Cytion catalog number 400107)
<b>Biosafety level</b>	1
<b>NCBI_TaxID</b>	10090
<b>CellosaurusAccession</b>	CVCL_0123
<b>GMO Status</b>	No genetic modification; 3T3-L1 is a subclone of the NIH/3T3 line selected for adipogenic differentiation potential; no introduced transgene

### Biomolecular Data

<b>Tumorigenic</b>	No
<b>Virus susceptibility</b>	Murine leukemia virus, murine sarcoma virus, vesicular stomatitis, vaccinia, herpes simplex, N-tropic oncornaviruses C
<b>Products</b>	Insulin, collagen, triglycerides
<b>Ploidy status</b>	Aneuploid
<b>Karyotype</b>	2n=40

### Handling

<b>Culture Medium</b>	DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 3.7 g/L NaHCO <sub>3</sub> , w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)
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## 3T3-L1 Cells | 400107

**Supplements** Supplement the medium with 10% FBS

**Dissociation Reagent** Accutase

**Subculturing** Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.

**Split ratio** 1 to 5

**Seeding density** 1 to  $3 \times 10^4$  cells/cm<sup>2</sup>

**Fluid renewal** Every 2 to 3 days

**Freeze medium** As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

## 3T3-L1 Cells | 400107

### Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below  $-150^{\circ}\text{C}$  to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a  $37^{\circ}\text{C}$  water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at  $300 \times g$  for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

### Incubation Atmosphere

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified atmosphere.

### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

### Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about  $-150$  to  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  is acceptable only as a short interim step before transfer to liquid nitrogen.

## Quality Control & Molecular Analysis

## 3T3-L1 Cells | 400107

### **Sterility**

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.