

**L6565 Cells | 305189****General information****Description**

L6565 cells were derived from pancreatic suspensions of splenocytes from L6565 leukemia mice. The chromosomal count ranged from 38 to 144. Electron microscopic observations revealed that the clonal L6565 cells had well-defined nuclei and an abundance of organelles and Class A and Class C viral particles in the cytoplasm. The oncogenes c-myc and c-fos were overexpressed in these cells. The L6565 cell clone is an RNA virus-containing lymphoblastic leukemia stem cell line. It has passed the mycoplasma detection test in this library.

The significance of the L6565 cell line lies in its provision of standardized experimental cell resources and associated technical support for research in the fields of life sciences and biotechnology. These cells can be crucial for understanding the molecular mechanisms of leukemia, particularly the role of viral particles and oncogene expression in leukemogenesis. Additionally, they serve as a valuable tool for drug testing and development, allowing researchers to explore potential therapeutic strategies for leukemia and other related disorders.

**Organism** Mouse

**Tissue** Peripheral blood

**Characteristics**

**Morphology** Lymphoblast

**Growth properties** Adherent and Suspension

**Regulatory Data**

**Citation** L6565 (Cytion catalog number 305189)

**Biosafety level** 1

**NCBI\_TaxID** 10090

**CellosaurusAccession** CVCL\_A9NB

**Biomolecular Data****Handling**

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**Culture Medium** DMEM:Ham's F12 (1:1), w: 3.1 g/L Glucose, w: 2.5 mM L-Glutamine, w: 15 mM HEPES, w: 0.5 mM Sodium pyruvate, w: 1.2 g/L NaHCO<sub>3</sub> (Cytion article number 820400a)

**Supplements** Supplement the medium with 10% FBS, 0.005 mg/mL insulin, 0.01 mg/mL human transferrin, 0.1 mM ethanolamine, 0.1 mM phosphoethanolamine, 25 nM selenium, 500 nM hydrocortisone, 0.005 mM forskolin, bovine pituitary extract (0.15 mg protein per ml)

**Subculturing** Gently homogenize the cell suspension in the flask by pipetting up and down, then take a representative sample to determine the cell density per ml. Dilute the suspension to achieve a cell concentration of  $5 \times 10^5$  cells/ml with fresh culture medium, and aliquot the adjusted suspension into new flasks for further cultivation.

**Fluid renewal** 2 to 3 times per week

**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.

### 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°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°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 x 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.

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**Incubation Atmosphere** 37°C, 5% CO<sub>2</sub>, humidified atmosphere.

**Flask Coating** None

**Shipping Conditions** Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately -78 °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 °C. Storage at -80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

**Quality Control & Molecular Analysis**

**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.