

**L6565 Cells | 305189**

**General information**

<b>Organism</b>	Mouse
<b>Tissue</b>	Peripheral blood

**Characteristics**

<b>Morphology</b>	Lymphoblast
<b>Growth properties</b>	Suspension

**Identifiers / Biosafety / Citation**

<b>Citation</b>	L6565 (Cytion catalog number 305189)
<b>Biosafety level</b>	1

**Expression / Mutation**

**Handling**

<b>Culture Medium</b>	DMEM:Ham's F12, w: 3.1 g/L Glucose, w: 1.6 mM L-Glutamine, w: 15 mM HEPES, w: 1.0 mM Sodium pyruvate, w: 1.2 g/L NaHCO3 (Cytion article number 820400a)
<b>Medium supplements</b>	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)
<b>Passaging solution</b>	The utilization of a passaging solution is not necessary when passaging cells that are cultured in suspension. The appropriate procedure is to dilute the cells in accordance with the indicated guidelines.
<b>Subculturing</b>	Resuspend cell suspension in the flask and take representative aliquote to count the cell number per ml. Dilute cell suspension to $1 \times 10^5$ cells/ml with fresh medium and transfer into new flasks.
<b>Split ratio</b>	1:2 to 1:4
<b>Fluid renewal</b>	2 to 3 times per week

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### Freeze medium

CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)

### Handling of cryopreserved cultures

L6565 cells are shipped in a deep-frozen state on dry ice. Upon receipt, confirm that the vial remains frozen. For storage, place the cryovial immediately at temperatures below -150 degrees. If you plan to culture the cells immediately, swiftly thaw the vial by shaking it in a 37 degrees water bath with clean water and an antimicrobial agent for 40-60 seconds. Remove the vial once a small ice clump persists, ensuring it remains cold. Proceed with all subsequent steps under aseptic conditions. In a sterile flow hood, disinfect the cryovial with 70% ethanol. Then, gently open the vial and transfer the cell suspension into a 15 ml centrifuge tube pre-filled with 8 ml of room temperature culture medium. Gently mix the cells. For cell separation, centrifuge at 300 x g for 3 minutes and dispose of the supernatant. Skipping centrifugation is optional, although any residual freezing medium should be removed after 24 hours. Resuspend the pellet gently in 10 ml of fresh culture medium and divide between two T25 culture flasks. Follow the subculture protocol for subsequent steps.

### Handling of proliferating cultures

One or two cell culture flasks come filled with cell culture medium. Collect the entire medium in a 50 ml centrifuge tube. Spin down the collected medium at 300 x g for 3 minutes to collect the cells which may have detached during transit. If a cell pellet is visible, resuspend the cells in 5 ml of cell culture medium and transfer to a T25 cell culture flask. Carefully add 5 ml of cell culture medium to each T25 cell culture flask. Examine cell morphology and confluency using a microscope. Finally, incubate the flasks at 37 degrees Celsius for at least 24 hours.

## Quality control / Genetic profile / HLA

### Sterility

Mycoplasma contamination is rigorously 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.