Suspension

Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ air atmosphere is recommended if the cell density of the culture as suggested under the subculture procedure.

Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination.

Incubate the flask in an upright position for several hours at 37°C. After the temperature has equilibrated, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save for reuse. Resuspend the cell pellet in 10 mL of this medium.

From this cell suspension remove a sample for a cell count and viability. Adjust the cell density of the suspension to 2 to 5 x 10⁶ viable cells/mL in the shipping medium.

Incubate the culture, horizontally, at 37°C in a 5% CO₂ air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.
Cultures can be maintained by addition of fresh medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 1 x 10^5 viable cells/mL. Maintain cultures at a cell concentration between 1 x 10^5 and 1 x 10^6 cells/mL. Do not allow the cell concentration to exceed 1 x 10^6 cells/mL.

**Medium Renewal:** Every 2 to 3 days

**Cryopreservation Medium**

Complete growth medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

**Comments**

L3T4 is expressed on mouse helper/inducer T cells and is analogous to the human Leu-3/T4 molecule. Tested and found negative for ectromelia virus (mousepox).

**References**

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

**Biosafety Level: 1**

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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