Suspension

Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the vial in the water bath for an extended period to prevent the contents from contacting the vial walls. To thaw the vial:

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the vial in the water bath for an extended period to prevent the contents from contacting the vial walls.

2. Transiently warm the vial to 56°C in a 56°C water bath. This will sometimes assist in the thawing process. The thawing time may vary from culture to culture. When thawing is complete, the vial will become liquid. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete growth medium and spin at approximately 125 x g for 5 to 7 minutes.

3. Resuspend cell pellet with the recommended complete growth medium (see the specific batch information for the recommended dilution ratio), and dispense into a 25 cm² or a 75 cm² culture flask. 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).

4. 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.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information), grown, and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any 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.

3. From this cell suspension remove a sample for a cell count and viability. Adjust the cell density of the suspension to 2-3 x 10^6 viable cells/ml in the shipping medium.

4. Incubate the culture, horizontally, at 37°C in a 5% CO₂ in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.

**Subculturing Procedure**

Cultures can be maintained by addition or replacement of fresh medium. Start cultures at 2 x 10^5 cells/mL and maintain between 1 x 10^5 and 1 x 10^6 cells/mL.

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

**Cryopreservation Medium**

Complete culture medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

**Comments**

These cells differentiate spontaneously into erythroblast-like cells. Macrophage-like differentiation can be induced with phorbol esters such as TPA (12-O-tetradecanoyl-phorbol-13-acetate) and PMA (phorbol myristic acid).

**References**

References and other information relating to this product are available online at [www.atcc.org](http://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.

**ATCC Warranty**

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Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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Please read this FIRST

Storage Temp.
liquid nitrogen
vapor phase

Biosafety Level
1

Intended Use

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

Complete Growth Medium

The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, ATCC 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum (ATCC 30-2020) to a final concentration of 10%.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: HEL 92.1.7 (ATCC® TIB-180™)