Prepare a 25 cm² or a 75 cm² culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents, the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.

2. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

3. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All operations from this point on should be carried out under strict aseptic conditions.

4. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge the cell suspension at approximately 125 x g for 5 to 7 minutes.

5. Discard the supernatant and resuspend the cells in fresh growth medium (see the batch-specific information for the recommended dilution ratio). Add this suspension to the prepared culture vessel.

6. Incubate the culture at 37°C in a suitable incubator.

7. A 5% CO₂/95% air atmosphere is recommended if using the medium described on this product sheet.
Handling Procedure for Flask Cultures

The flask was seeded with cells, incubated, 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. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached**, aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C with 5% CO₂ until they are ready to be subcultured.
3. **If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 125 g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm² flask. Incubate at 37°C with 5% CO₂ until cells are ready to be subcultured.

Population Doubling Capacity

As part of our quality control, we have tested this cell line for its ability to grow for a minimum of 15 population doublings after recovery from cryopreservation. We have also compared its karyotype, telomerase expression level, growth rate, morphology and tissue-specific markers when first recovered from cryopreservation with that of cells at 10+ population doublings to ensure that there is no change in these parameters and that the cells are capable of extended proliferation.

Subculturing Procedure

Volumes are given for T-75 flasks; proportionally reduce or increase the amount of dissociation medium for culture vessels of other sizes.
1. Remove and discard culture medium.
2. Add 2.0-3.0 mL of 0.05% Trypsin-0.53 mM EDTA solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 to 10 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
3. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
4. Transfer cell suspension to a centrifuge tube and spin at approximately 125 x g for 5 to 15 minutes.
5. Discard supernatant and resuspend cells in fresh growth medium.
6. Add appropriate aliquots of cell suspension to new culture vessels. An inoculum of 2 X 10^2 to 3 X 10^3 viable cells/cm² is recommended. Subculture when cell concentration reaches between 1 x 10^4 and 2 x 10^4 cells/cm².
7. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:6 is recommended

Cryopreservation Medium

90% heat-inactivated fetal bovine serum and 10% DMSO.

Store in liquid nitrogen vapor. Avoid immersing vials into liquid nitrogen.

References

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

Biosafety Level: 2

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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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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