Suspension

Biosafety Level

Incubate the culture at

Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by

Resuspend cell pellet with the recommended complete medium (see the specific batch information for

It is important to note that some vials leak when submersed in liquid nitrogen

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

Check all containers for leakage or breakage.

Incubate the culture, horizontally, at 37°C in a 5% CO

Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium. and spin at

Incubate the flask in an upright position for several hours at 37°C. After the temperature has

Thaw the vial by gentle agitation in a

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SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be

Unpacking & Storage Instructions

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature
below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If
upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor
phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. 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).
2. 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 of the operations from this point on should be carried out
under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium. and spin at
approximately 125 x g for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for
the culture 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).
5. 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.
Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for
any evidence of microbial contamination
2. 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. 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 of fresh medium or replacement of medium. Alternatively, cultures can
be established by centrifugation with subsequent resuspension in fresh medium.
Add fresh medium as cell density increases.
Cells grow as floating aggregates of round cell clusters.
Medium Renewal: Every 3 to 5 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.

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

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