Unpacking & Storage Instructions

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

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 until -130°C, preferably in liquid nitrogen vapor, until ready for use.

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 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. 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 32°C in a 5% CO₂ in air atmosphere 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 x 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 32°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.
Subculturing Procedure

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.
1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-053 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 32°C.

Subcultivation Ratio: 1:3 to 1:4

Medium Renewal: Every 2 to 3 days

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in *Culture of Animal Cells, a Manual of Basic Technique* by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

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

The 15P-1 cell line was established from testicular cells of transgenic mice that express the large T protein of polyoma virus (PyLT) in the seminiferous epithelium. Ref
This line exhibits characteristics of Sertoli cells, including transcription of the Wilms' tumor (WT1) and Steel genes. Ref
15P-1 cultures express phagocytic activity evidenced by the internalization of latex beads and the disposal of dead cells. Interaction with germ cells enhances the phagocytic activity of 15P-1 cells. Ref
The cells support the meiotic and postmeiotic differentiation in cocultures of diploid premeiotic germ cells into haploid spermatids expressing the protamine (Prm-1) gene. Ref
When cocultured with 15P-1 cells, testicular cells explanted from immature 9-day-old animals, before the onset of the first meiosis, generate tetrads of haploid cells with the morphology of round spermatids and initiate protamine transcription. Ref
The 15P-1 cell line releases protease-sensitive material with broad-spectrum antibacterial activity. The activity of 15P-1 culture medium is increased 10-fold to 50-fold in the presence of fractions enriched in round spermatids and of nerve growth factor. Ref
The KL protein, a ligand of the Kit receptor, is expressed in Sertoli cells and in 15P-1 cells. Ref

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.

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

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