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

These cells are grown in:
- 50 % L-15 (ATCC 30-2008)
- 35 % DMEM HG (GIBCO 12100)
- 15 % Ham's F12 (GIBCO 21700)

All without sodium bicarbonate Supplemented with:
- 0.15 g/L sodium bicarbonate
- 15 mM HEPES
- 0.01mg/ml bovine insulin
- 50 mg/ml mouse EGF
- 5% heat-inactivated fetal bovine serum
- 0.5% Trout Serum

Do not filter complete medium.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: ZFL [ZF-L] (ATCC® CRL-2643™)

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 28°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. It is recommended that the cryoprotective agent be removed immediately. Resuspend content of ampoule in 9 mL of complete growth medium containing an additional 5% heat-inactivated FBS. Centrifuge the cell suspension at approximately 125 x g for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of serum-free growth medium.
4. Transfer the vial contents to an appropriate size vessel. Incubate the culture at 28°C in a suitable incubator for 30 mins. If using the medium described on this product sheet, the medium formulation was devised for use in a free gas exchange with atmospheric air. A CO₂ and air mixture is detrimental to cells when using this medium for cultivation.
5. Examine to ensure attachment and then add heat-inactivated FBS for a final concentration of 5% and Trout serum for a final concentration of 0.5%.

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 28°C 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...
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% (v/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that 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).
   **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.
4. Add 6.0 to 8.0 mL of complete growth medium containing 10% heat-inactivated FBS and aspirate cells by pipetting gently.
5. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
6. Discard supernatant and resuspend cells in fresh serum-free growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
7. Place culture vessels in incubators at 28°C for 30 minutes.
8. Examine to ensure attachment, and then add heat-inactivated FBS for a final concentration of 5% and Trout serum for a final concentration of 0.5%.

Subcultivation Ratio: 1:2 to 1:3

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

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

Comments

The cells synthesize and release several proteins into the culture medium, including a 70 kDa protein recognized by anti-bovine serum albumin IgG.

A culture submitted to the ATCC in August of 2001 was found to be contaminated with mycoplasma. Progeny were cured by a 21-day treatment with BM Cycline. The cells were assayed for mycoplasma, by the Hoechst stain, PCR and the standard culture test, after a six-week period following treatment. All tests were negative.

The dioxin, TCDD, induces one or possibly two forms of cytochrome P450 immunochemically and functionally related to rainbow trout P4501A1. ZFL cell homogenates exhibit alanine and aspartate aminotransferase, glucose-6-phosphatase and alkaline phosphatase enzyme activities.

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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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

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

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