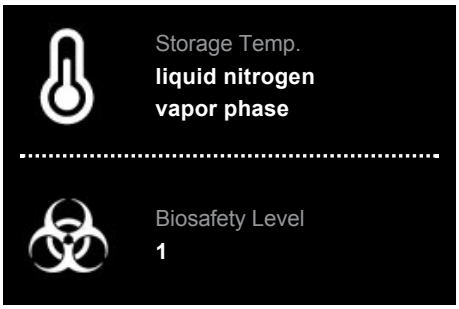
ATCC

Product Sheet

ZFL [ZF-L] (ATCC® CRL-2643™)

Please read this FIRST



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 ng/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™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Danio rerio*, zebrafish
Tissue: liver
Disease: Normal
Age: adult
Morphology: epithelial
Growth Properties: adherent
Cytogenetic Analysis: hypodiploid; modal number = 47

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

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.

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 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 ampule 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 CO_2 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

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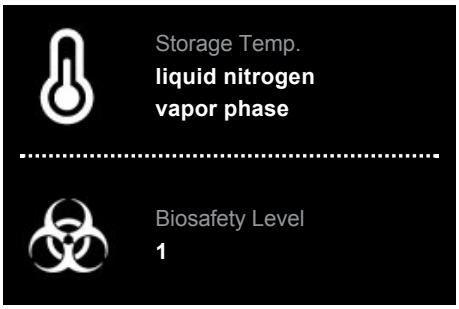

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125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm^2 flask. Incubate at 28°C in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 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-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.

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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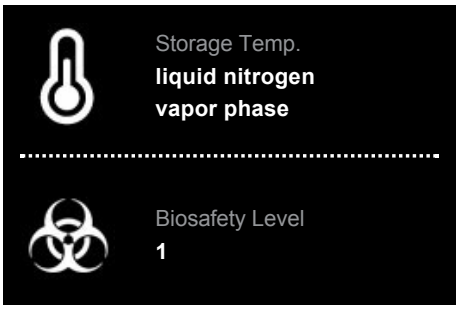

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Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.
While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.
This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.
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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