ZFL [ZF-L]

CRL-2643™

Description

Organism: *Danio rerio*, zebrafish
Tissue: Liver
Age: adult
Morphology: epithelial
Growth properties: Adherent
Disease: Normal

Storage Conditions

Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 28°C (26-29°C)
Atmosphere: 100% Air

Handling Procedures

Unpacking and 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.

Complete 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
- 10 µg/ml human insulin
- 50 ng/ml mouse EGF
- 5% heat-inactivated fetal bovine serum
- 0.5% Trout Serum

Note: Trout serum *must* be sterile filtered before addition to the culture media and application to the cells. Do not filter complete medium.

Media preparation instructions:

- 500 mL DMEM/F-12lL-15 modified for CRL-2643 (includes 0.15 g/L sodium bicarbonate, 0.15 g/L sodium bicarbonate, 10 µg/ml human insulin)
- 28 mL heat-inactivated FBS (ATCC 30-2020)
- 2.8 mL Trout Serum (Caisson catalog # TSL011oOML / Fisher Scientific catalog # NC0790887. or equivalent)
  Note: The above prepared medium is stable for 1 month when stored at 2.0 to 8.0°C.

Due to limited stability, the following component should be added to an aliquot of the above culture medium fresh prior to seeding or performing fluid changes. Complete media supplemented with the mouse EGF expires 7 days after preparation.

Add 1.9 uL per 1 mL culture medium.

- To prepare 26.6 ug/mL EGF stock solution, aseptically combine the following:

Note: To prepare a 26.6 pg/mL stock of EGF, aseptically combine the following:

- 1 vial of 0.1 mg Mouse EGF (Sigma catalog # E1257 or equivalent)
- 3.76 mL PBS with 0.1% BSA or 3.76 mL culture medium (excluding EGF), see above.

Note: Stock EGF can be stored at 2 to 8°C for 14 days, or aliquots of EGF can be stored at -20°C for up to 2 months. Avoid freeze-thawing

Handling Procedure: To ensure 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 280 x *g* for 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of serum-free growth medium (see additional comments below).

   Comments (additional handling instruction):

   - Following centrifugation at both startup and subcultures resuspend the pellet into culture medium (without serum). (see the lot information on Certificate of Analysis (COA) for the culture recommended dilution ratio) and dispense into a 25 cm^2 or a 75 cm^2 culture flask as recommended on the COA.
   - Transfer the cell suspension to the appropriate vessel(s) and incubate for ~30 minutes (or until the cells attach).
   - After the cells attach, add heat-inactivated FBS and trout serum to the vessel(s) to achieve the appropriate final concentrations (5% and 0.5% respectively)-

4. Transfer the vial contents to an appropriate size vessel. A non-vented flask is recommended. The recommended seeding density for CRL-2634 is 3.0 x 10^4 to 8.0 x 10^4 viable cells/cm^2. 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%.

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/ Subculture seeding density: A subcultivation ratio of 1:2 to 1:3 is recommended or subculture at a seeding density of 3.0 x 10^4 to 6.0 x 10^4 viable cells/cm^2.
Medium Renewal: Every 2 to 3 days.
Freeze Culture Medium: Complete Culture Medium + additional 4.5% heat-inactivated
FBS + 5% DMSO (ATCC 4-X)
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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: ZFL [ZF-L] (ATCC CRL-2643)

References

References and other information relating to this material are available at www.atcc.org.

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