Fugu eye

CRL-2641™

Description

Organism: *Fugu rubripes*, torafugu
Tissue: Eye
Age: juvenile
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: 22°C (21-23°C)

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

- 67.5% DMEM HG without sodium bicarbonate (Invitrogen Cat no. 12100)
- 25% L-15 (ATCC Cat no.30-2008)
- 7.5% Ham?s F12 without sodium bicarbonate (Invitrogen Cat no. 21700)

Supplemented with:

- 0.5 g/L sodium bicarbonate
- 15 mM HEPES
- 0.01 mg/ml bovine insulin
- 0.05 mM 2-mercaptoethanol
- 1.0 mM L-glutamine
- 0.05 mM non-essential amino acids
- 10 ng/ml basic human recombinant FGF
- 50 ng/ml mouse EGF (Do not filter)
- 5 to 7.5% heat-inactivated fetal bovine serum

Handling Procedure:

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.

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.

1. Thaw the vial by gentle agitation in a **21 to 23°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. Centrifuge the cell suspension at approximately 125 xg for 5 to 10 minutes. Discard

the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium.

4. Transfer the vial contents to an appropriate size vessel.

5. Incubate the culture at **21 to 23°**C in a suitable incubator. 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.

Subculturing procedure:
Protocol:

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 21 to 23°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 xg for 5 to 10 minutes.
6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
7. Incubate cultures at 21 to 23°C without CO2.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:4 is recommended; cell density must be high.
Medium Renewal: Every 2 to 3 days
Reagents for cryopreservation: Complete growth medium supplemented with 5% (v/v) DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the

following manner: Fugu eye (ATCC CRL-2641)

References

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

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