Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
2

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
Dulbecco’s modified Eagle’s medium with 4 mM L-glutamine adjusted to contain 1.5g/L sodium bicarbonate and 4.5 g/L glucose and supplemented with 0.1 mM non-essential amino acids, 96%; fetal bovine serum, 4%

Citation of Strain
If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: RPE-J (ATCC® CRL-2240™)

Description
Organism: Rattus norvegicus, rat
Strain: Long-Evans
Tissue: eye; retinal pigmented epithelium; retina
Disease: normal
Cell Type: epithelial SV40 transformed
Age: 7 days
Morphology: epithelial
Growth Properties: adherent

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.

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. 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 33°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
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-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 and aspirate cells by gently pipetting.
5. Transfer cell suspension to a centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes. Discard supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
7. Place culture vessels in incubators at 33°C.

Subculture Ratio: 1:3
Medium Renewal: Every 2 to 3 days.


Cryopreservation Medium

Complete growth medium (see above) with an additional 16% fetal bovine serum and 5% (v/v) DMSO.

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

Comments

The cells express a transformed phenotype at the permissive temperature (33°C), and a non-transformed phenotype at the non-permissive temperature (40°C). They must be cultured at the permissive temperature and do not grow at 37°C.

When RPE-J cells are grown on nitrocellulose filters coated with a thin layer of Matrigel in the presence of 10(-8) M retinoic acid for 6 days at 33°C and then switched to the non-permissive temperature of 40°C for 33 to 36 hours, they acquire a differentediated polarized RPE phenotype. Under these conditions, RPE-J cells exhibit circumferential staining for the tight-junction protein ZO-1 and acquire a transepithelial resistance of 350 ohms/cm². Ref

RPE-J is the only established RPE cell line that maintains epithelial cell surface polarity. The cells retain many properties of RPE including expression of the rat RPE marker RET-PE2 and the ability to phagocytose latex beads. Ref

A culture submitted to the ATCC in July 1995 was found to be contaminated with Mycoplasma hyorhinis and was cured by a 21-day treatment with BM Cycline. The cells were assayed for mycoplasma by the Hoechst stain and the standard culture test over a six-week period following treatment and all tests were negative. RPE-J is a retinal pigment epithelial (RPE) cell line derived from primary cultures of RPE cells taken from 7-day-old Long-Evans rats.

References

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