



RWPE2-W99

CRL-2853™

Description

Cell Type: epithelial cell

Age: 54 years

Gender: Male

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 2

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.

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Cells contain Human papillomavirus type 18 (HPV-18) sequences

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: 37°C

Atmosphere: 95% Air, 5% CO₂

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: The base medium for this cell line is provided by Invitrogen (GIBCO) as part of a kit: Keratinocyte Serum Free Medium (K-SFM), Kit Catalog

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Number 17005-042. This kit is supplied with each of the two additives required to grow this cell line (bovine pituitary extract (BPE) and human recombinant epidermal growth factor (EGF). To make the complete growth medium, you will need to add the following components to the base medium:

0.05 mg/ml BPE - provided with the K-SFM kit

5 ng/ml EGF - provided with the K-SFM kit. NOTE: Do not filter complete medium.

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 37°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. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately $125 \times g$ for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm^2 or a 75 cm^2 culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product.

Subculturing procedure:**Protocol:**

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca⁺⁺/Mg⁺⁺ free Dulbecco's phosphate-buffered saline (D-PBS).
3. Add 2.0 to 3.0 ml (to a T-25 flask) or 3.0 to 4.0 ml (to a T-75 flask) of 0.05% Trypsin - 0.53 mM EDTA solution, diluted 1:1 with D-PBS, and place flask in a 37C incubator for 5 to 8 minutes. Observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 10 minutes).
Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach.
4. Add 6.0 to 8.0 ml of 0.1% Soybean Trypsin Inhibitor (or 2% fetal bovine serum in D-PBS), as appropriate, and aspirate cells by gently pipetting.
5. Transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 7 minutes.
6. Discard supernatant and resuspend cells in fresh serum-free growth medium. Add appropriate aliquots of cell suspension to new culture vessels. An inoculum between 2 X 10⁽⁴⁾ to 4 X 10⁽⁴⁾ viable cells/sq. cm is recommended.
7. Incubate cultures at 37C. We recommend that you maintain cultures at a cell concentration between 4 X 10⁽⁴⁾ and 8 X 10⁽⁴⁾ cells/sq. cm.

Cells grown under serum-free or reduced serum conditions may not attach strongly during the 24 hours after subculture and should be disturbed as little as possible during that period.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:5 is recommended

Medium Renewal: Every 48 hours

Note: Subculture cells before they reach confluence. Do not allow cells to become confluent.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: RWPE2-W99 (ATCC CRL-2853)

References

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

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