



VK2/E6E7

CRL-2616™

Description

VK2/E6E7 is an epithelial cell line that was isolated in 1996 from the mucosa of a 32-year-old, female patient undergoing a hysterectomy for endometriosis. This cell line was deposited by D Anderson, RN Fichorova, and JG Rheinwald.

Organism: *Homo sapiens*, human

Cell Type: epithelial cell

Tissue: Vagina; Mucosa

Age: 32 years

Gender: Female

Morphology: epithelial

Growth properties: Adherent

Disease: Endometriosis

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

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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.

Cells contain Human papillomavirus (HPV) 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: Keratinocyte-Serum Free medium (GIBCO-BRL 17005-042) with 0.1 ng/ml human recombinant EGF, 0.05 mg/ml bovine pituitary extract, and additional calcium chloride 44.1 mg/L (final concentration 0.4 mM)

Handling Procedure: 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 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. It is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium. Centrifuge the cell suspension at approximately $125 \times g$ for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium.
4. Transfer the cells an appropriate size vessel. 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 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% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

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.

Note: The cells should not be allowed to become confluent, subculture at 60 to 90%

of confluence.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.03% (w/v) 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. Neutralize the trypsin by adding 6.0 to 8.0 ml of a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium (DMEM:F-12, ATCC Catalog No. 30-2006), containing 10% fetal bovine serum. Aspirate cells by gently pipetting.
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 37°C. Cells will not attach well for 24 hours after subculture.

Subcultivation ratio: 1:3 to 1:5

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

Reagents for cryopreservation: A 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium supplemented with 10% (v/v) fetal bovine serum and 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: VK2/E6E7 (ATCC CRL-2616)

References

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

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Revision

This information on this document was last updated on 2023-01-07

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