**Product Sheet**

**VK2/E6E7 (ATCC® CRL-2616™)**

**Description**

- **Organism:** Homo sapiens, human
- **Tissue:** vagina, mucosa
- **Disease:** Endometriosis
- **Cell Type:** epithelial-HPV-16 E6/E7 transformed
- **Age:** 32 years adult
- **Gender:** female
- **Morphology:** epithelial
- **DNA Growth Properties:** adherent

**DNA Profile:**
- Amelogenin: X
- CSF1PO: 10, 11
- D5S818: 9, 10
- D13S317: 9, 12
- D7S820: 10, 11
- D16S539: 9
- VWA: 16
- THO1: 7, 9.3
- TPOX: 11

**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.

**Unpacking & 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.

**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. 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 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 cells to 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% CO₂ in air atmosphere is recommended if using
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 37°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 ml of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Protocol: The cells should not be allowed to become confluent, subculture at 60 to 90% of confluence. Remove medium, and rinse with 0.25% trypsin, 0.53mM EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37°C) until the cells detach. Neutralize the trypsin by adding a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium containing 10% fetal bovine serum. Centrifuge the cell suspension at 1000 rpm for 10 minutes, resuspend the pellet in fresh serum-free growth medium, aspirate and dispense into new flasks. Cells will not attach well for 24 hours after subculture.

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

Medium Renewal: Every 2 to 3 days

Cryopreservation Medium

A 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium, 85%; fetal bovine serum, 10%; DMSO, 5%

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

Comments

Cells at passage 3 were immortalized by transduction with the retroviral vector LXSN-16E6E7 in the presence of polybrene. Clones were selected in medium containing G418.

The endocervical cell line expresses characteristics of simple columnar epithelium, whereas the ectocervical and vaginal cell lines express characteristics of stratified squamous nonkeratinizing epithelia. Without stimulation, all three cell lines produce macrophage colony-stimulating factor (M-CSF), transforming growth factor beta1, interleukin 8 (IL-8), prostaglandin E2, the secretory leukoprotease inhibitor, and the polymeric immunoglobulin receptor. The endocervical cell line (End1/E6E7), but not the others, also produce the lymphopoietic cytokines IL-6, IL-7, and consistently detectable levels of the chemokine known as "regulated-upon-activation, normal T cell expressed and secreted" (RANTES).

Stimulation with interferon gamma and tumor necrosis factor alpha (TNF alpha) induces or significantly up-regulates expression of several of the cytokines and chemokines as well as major histocompatibility complex (MHC) class II antigens in the lines. Pililiated, but not nonpiliated, Neisseria gonorrhoea strain F62 variants actively invade these epithelial cell lines. Invasion of these cells by green fluorescent protein-expressing gonococci is characterized by colocalization of gonococci with F actin.

These cell lines may provide the basis for valid, reproducible in vitro models for studies on cervicovaginal physiology and infections and for testing pharmaceutical agents for intravaginal application.

References

References and other information relating to this product are available online at [www.atcc.org](http://www.atcc.org).

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in...
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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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