A2EN Human Endocervical Epithelial Cell Line

Immortalized Cell Line
Cat. # SCC131

Pack size: ≥1X10^6 viable cells/vial

Store in liquid nitrogen

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.
THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS.

Data Sheet

Background

The endocervical epithelium is a single cell layer lining the endocervical canal that separates the lower female reproductive tract from the uterus and Fallopian tubes. Endocervical epithelial cells secrete mucus and innate immune mediators that facilitate reproduction as well as protect from pathogen invasion. The endocervix is a major site of transmission for multiple sexually transmitted organisms, such as *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium* and human immunodeficiency virus (HIV).

A2EN is an established *in vitro* cell model for the study of sexually transmitted pathogen infections, pathogenesis and the innate immune response. A2EN is an immortalized human epithelial cell line derived from human primary epithelial cells expanded from an explant of human endocervical tissue. The primary endocervical epithelial cells were immortalized with lentiviral vectors carrying the HPV E6/E7 genes. Passages >45 can generate a transepithelial electrical resistance (TEER) when grown on cell culture inserts and generate differential apical and basolateral cytokine profiles upon stimulation with various toll-like receptor (TLR) agonists.

A2EN human endocervical epithelial cells are adherent and exhibit a keratinocyte appearance when cultured on plastic and a cobblestone appearance with occasional multilayer regions when grown on cell culture inserts.

Short Tandem Repeat (STR) Profile

D3S1358: 15	D16S539: 9, 11
TH01: 6	CSF1PO: 12, 13
D21S11: 29, 32.2	Penta D: 9, 12
D18S51: 14, 18	vWA: 17
Penta E: 7, 10	D8S1179: 12, 16
D5S818: 11, 13	TPOX: 8, 11
D13S317: 9, 13	FGA: 21
D7S820: 11	Amelogenin: X

Immortalized cell lines are inherently genetically unstable. Genetic instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

Storage and Handling

A2EN Human Endocervical Epithelial Cell Line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are tested negative for Epstein-Barr virus, HPV-16, HPV-18, Hepatitis A, C, Herpesvirus type 6, 7, 8 and HIV-1 & 2 viruses by PCR
- Cells are negative for mycoplasma contamination.
- Each lot of cells is genotyped by STR analysis to verify the unique identity of the cell line.

Representative Data

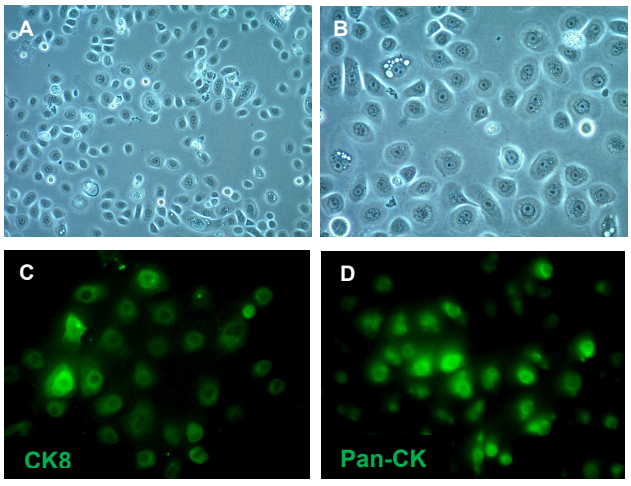

Figure 1. Day 1 after thaw (**A**, 10X mag; **B**, 20X mag). A2EN express epithelial cytokeratin CK8 (**C**) and pan cytokeratin (**D**) markers.

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

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Protocols

Appropriate growth of A2EN cells requires that the media used contain calcium concentrations ranging from 0.06 mM – 0.4 mM. Calcium concentration of Serum-free EpiGRO Human Epidermal Keratinocyte Media (Cat. No. SCMK001) is 0.06 mM.

Phenol-red free media is optimal for performing hormone experiments using A2EN since phenol red may interfere with hormone receptors. A2EN cells are typically grown without the use of antibiotics, however, standard penicilliln/streptomycin additives can be added and do not impair cell growth. While A2EN cells are grown under serum-free conditions, FBS is used during both the passaging to inactivate the trypsin and also should be included in the cryopreservation medium.

Thawing Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue cultureware surfaces without any additional coating.

   Cells are thawed and expanded in Serum Free EpiGRO Human Epidermal Keratinocyte Media (Cat. No. SCMK001) supplemented with 5 ng/mL EGF (Sigma Cat. No. E9644) and 1X Penicillin-Streptomycin Solution (Cat. No. TMS-AB2-C, optional).
2. Remove the vial of frozen A2EN cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of A2EN Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 10-15 mL of A2EN Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37°C in a humidified incubator with 5% CO_2.
12. The next day, exchange the medium with 10-15 mL of fresh A2EN Expansion Medium. Exchange with fresh medium every two to three days thereafter.
13. When the cells are approximately 85-90% confluent, they can be dissociated with Trypsin/EDTA (Sigma T3924) and further passaged or, alternatively, frozen for later use. Typical split ratio is 1:3.

    Do not use Accutase or Accumax as either may result in insufficient cell detachment.

Subculturing Cells

1. Carefully remove the medium from the T75 tissue culture flask containing the 85-90% confluent layer of A2EN cells.
2. Rinse the T75 flask twice with 10 mL 1X PBS. Aspirate after each rinse.
3. Apply 3-5 mL of trypsin-EDTA solution (Sigma T3924) and incubate in a 37°C incubator for 3-5 minutes.
4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
5. Add 8 mL of **A2EN Expansion Medium supplemented with 10% FBS** to the plate. Addition of FBS is needed to inactivate the trypsin.
6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
7. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
8. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
9. Apply 2 mL of A2EN Expansion Medium (No FBS) to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
10. Count the number of cells using a hemocytometer.
11. Plate the cells to the desired density. Typical split ratio is 1:3.

Cryopreservation of Cells

A2EN Human Endocervical Epithelial Cell Line may be frozen in the expansion medium plus 10% FBS and 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

References

1. Herbst-Kralovetz MM, Quayle AJ, Ficarra M, Greene S, Rose WA 2nd, Chesson R, Spagnuolo RA, Pyles RB (2008) Quantification and comparison of toll-like receptor expression and responsiveness in primary and immortalized human female lower genital tract epithelia. *Am J Reprod Immunol* 59(3): 212-224.
2. Buckner LR, Schust DJ, Ding J, Nagamatsu T, Beatty W, Chang TL, Greene SJ, Lewis ME, Ruiz B, Holman SL, Spagnuolo RA, Pyles RB, Quayle AJ (2011) Innate immune mediate profiles and their regulation in a novel polarized immortalized epithelial cell model derived from human endocervix. *J Reprod Immunol* 92(1-2): 8-20.

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