Data Sheet

12Z Human Endometrial Epithelial Cell Line

Immortalized Cell Line

SCC443

Pack Size: ≥1x10^6 viable cells/vial

Store at: Liquid nitrogen

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Background

Endometriosis is a gynecologic condition that is characterized by presence of endometrial tissue outside of the uterus.[1] This condition affects around 6-10% of all women and could lead to pelvic pain and infertility.[2] The limited availability of in vitro models hinders cellular, molecular, and pathophysiologic studies on endometriosis which translates into the poor understanding of the disease and limited available treatments.[3]

Several endometriotic cell lines were developed by researchers from Goethe University Frankfurt in Germany.[3] One of these is the 12Z cell line which was derived from the endometrial peritoneum of a 37-year-old female undergoing laparoscopy. The 12Z cell line was immortalized using SV40 plasmid transfection. The cell line expresses both pan-cytokeratin and N-Cadherin.

One of the major models for the initiation of endometriosis is retrograde menstruation which then allows for endometrial tissues to be deposited in various tissues outside of the endometrium.[3] During this process, the cell's invasive property is thought to be essential. The 12Z immortalized human endometrial epithelial cell line is an established model for endometriosis being derived from an N-Cadherin+ endometriotic tissue, a marker for invasiveness. Moreover, this property of the cell line allows it to be used for various studies related to disease progression, pathogenesis, or therapeutics. Commonly, the 12Z cell line is used in 2D[4] or 3D[5] invasion assays and biochemical assays to study the pathophysiology of endometriosis.

Source

12Z cell line was derived from endometrial peritoneum biopsy of a 37-year-old female. This cell line was immortalized via transfection of a plasmid containing SV40T.

Storage and Handling

12Z cells should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting functionality.

Short Tandem Repeat (STR) Profile

D3S1358: 14, 17	D21S11: 28, 30.2	TH01: 9, 9.3	Penta E: 11, 12
D7S820: 9, 12	D18S51: 13, 15	TPOX: 11, 12	
vWA: 17	D5S818: 12	CSF1PO: 11, 13	
FGA: 22, 23	D13S317: 12, 13	AMEL: X	
D8S1179: 10	D16S539: 13	Penta D: 9, 12	

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

Quality Control Testing

- Each vial contains ≥ $1x10^6$ viable cells.
- Cells are tested negative for infectious diseases by a Human Essential CLEAR Panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of human origin and negative for inter-species contamination from mouse, rat, Chinese hamster, Golden Syrian hamster, and Non-human Primate (NHP) as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.

Representative Data

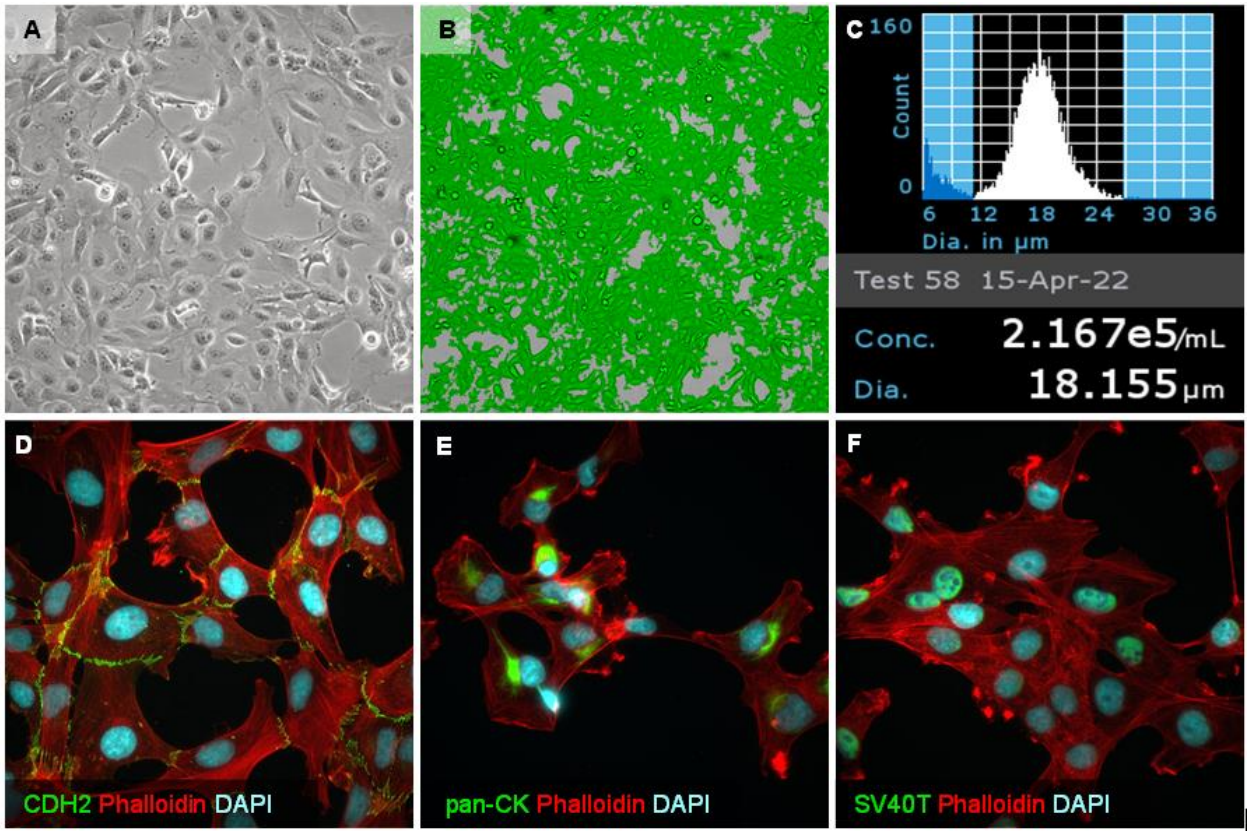

Figure 1. Bright-field image of 12Z cells two days after thaw in a T75 flask (**A**). Cell confluency (82%) was assessed throughout the culture using MilliCell® Digital Cell Imager (Cat. No. MDCI 10000) (**B**). Cell counting was performed using Scepter™ 3.0 handheld automated cell counter (PHCC360KIT) using 60 mm sensor tips (**C**). 12Z cells express the invasive marker CDH2 (N-Cadherin) (**D**), general epithelial marker, pan-cytokeratin (**E**), and immortalization antigen SV40T (**F**).

Protocols

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 12Z Expansion Medium comprised of DMEM medium (Cat. No. SLM-120-B) with 10% FBS (Cat. No. ES-009-B), 2 mM L-Glutamine (Cat. No. TMS-002-C), and 1X penicillin/streptomycin (Cat. No. TMS-AB2-C, optional).
2. Remove the vial of frozen 12Z 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 12Z 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 1100 rpm for 5 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 20 mL of 12Z 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.

Subculturing Cells

1. 12Z cells should be passaged at ~80-85% confluency. Do not allow the cells to grow over 85% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of 12Z cells. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
3. Apply 5 mL of Accutase™ or Trypsin/EDTA solution and incubate in a 37 °C incubator for 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 12Z Expansion Medium to the plate.
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.
9. Add 2-5 mL of 12Z Expansion Medium to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
10. Count the number of cells using a hemocytometer or a Scepter™ 3.0 handheld automated cell counter using 60 mm sensor tips.
11. Plate the cells to the desired density. Typical split ratio is 1:5 to 1:8. The medium should be replaced every other day.

Cryopreservation of Cells

12Z cells may be frozen in 12Z Expansion Medium and 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Nat. Rev. Endocrinol 2019, 15(11): 666-682.
2. Lancet 2004, 364 (9447): 1789-1799.
3. Am. J. Pathol 2001, 159(5): 1839-1852.

Reproductive BioMedicine Online 2022, 44(6): 976-990. DOI: https://doi.org/10.1016/j.rbmo.2022.02.008.

5. Sci Rep 2021, 11(1): 4115.

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