Data Sheet

OVCAR-5 Human Cancer Cell Line

Cancer Cell Line

SCC259

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.

Background

Ovarian carcinoma is a prevalent worldwide disease, with over 200,000 new cases diagnosed per year.[1] Metastatic gastrointestinal cancer frequently presents as advanced ovarian carcinoma.[2] The availability of cellular models that recapitulate the spectrum of forms of ovarian cancer are essential to understanding drug resistance, cellular physiology, and advancing new options for treatment.

The OVCAR-5 cell line was established from ascites fluid from a non-treated patient with an advanced-stage ovarian tumor.[3] The OVCAR-5 cell line has recently been classified as originating from the gastrointestinal tract, not of ovarian origin.[4] OVCAR-5 cells harbor a homozygous Gly12Val mutation in the KRAS oncogene and are characterized by migration/invasion ability as well as exhibiting tumorigenicity in nude mice.[5] OVCAR-5 cells overexpress claudin-4, a marker that plays a role in cancer malignancy.[6] OVCAR-5 cells exhibit aggressive growth and invasion and represent a valuable model for metastatic gastrointestinal carcinoma.

Source

The OVCAR-5 cells was established from ascites fluid from a 67-year-old untreated cancer patient.[3]

Short Tandem Repeat

D3S1358: 15, 16	D16S539: 11
TH01: 7, 9.3	CSF1PO: 10
D21S11: 31	Penta D: 12
D18S51: 12	vWA: 16
Penta E: 9, 16, 17	D8S1179: 13,14
D5S818: 12, 13	TPOX: 8, 11
D13S317: 10, 13	FGA: 23
D7S820: 10	Amelogenin: X

Cancer 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.

Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells tested negative for infectious diseases using 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 using a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.

Storage and Handling

The OVCAR-5 cells 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.

Representative Data

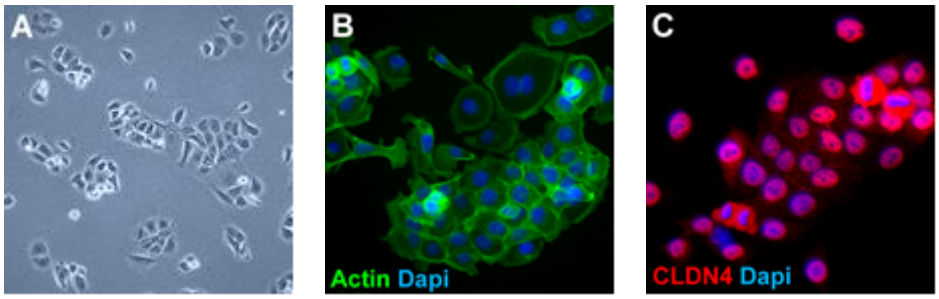

Figure 1. (**A**) Bright-field image of OVCAR-5 cells one day after thaw. (**B**) OVCAR-5 cells express actin (P5282) and (**C**) Claudin-4 (SAB4200574).

Protocols

Thawing of 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.

   OVCAR-5 Expansion Medium: Cells are thawed and expanded in OVCAR-5 Expansion Medium comprised of RPMI-1640 (R8758) supplemented with 2 mM glutamine (TMS-002-C), 10% FBS (ES-009-B), 0.25 U/mL insulin (407709), and 1X Penicillin/Streptomycin (TMS-AB2-C).

   Note: Insulin (407709) is insoluble from pH 4.5-7.0 and must be adjusted with HCl to pH 2-3 in order to dissolve. Below is the protocol to dissolve the insulin:

   - To make a 10 mg/mL stock solution, add 5 mL sterile water to the 50 mg bottle.
   - Add 0.1 N HCl dropwise while mixing until the insulin powder is dissolved.
   - Check that the pH is between 2-3.
   - Filter the solution though a 0.2 µM filter and store at 2-8 °C.
2. Remove the vial of frozen OVCAR-5 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 OVCAR-5 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 15 mL of OVCAR-5 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 of Cells

1. Do not allow the cells to grow to confluency. OVCAR-5 cells should be passaged at ~80-85% confluence.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of OVCAR-5 cells. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
3. Apply 5-7 mL of Accutase® 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 5-7 mL of OVCAR-5 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, then loosen the cell pellet by tapping the tip of the tube with a finger.
9. Apply 2-5 mL of OVCAR-5 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.
11. Plate the cells to the desired density. Recommended split ratio is 1:5.

Cryopreservation of Cells

The OVCAR-5 Human Cancer Cells may be frozen in OVCAR-5 Expansion Medium and 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Nat Rev Disease Primers 2016; 2: 16061.
2. J Obstet Gynaecol Can 2003; 25(10): 819-824.
3. Cancer Res 1997; 57: 850-856.
4. Nuc Acids Res 2016;44(17): e137.
5. Gynecol Oncol 2015;138(2): 372-377.
6. Int J Mol Sci 2011; 12: 1334-1358.

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