

Data Sheet

# OVCAR-3 Human Ovarian Cancer Cell Line

#### **SCC257**

Pack Size ≥ 1x10<sup>6</sup> 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. The first-line chemotherapy for newly-diagnosed ovarian carcinoma is cisplatin, but despite high initial efficacy, the most common types of ovarian carcinomas frequently relapse into platinum-resistant forms and many demonstrate multi-drug resistance. The availability of cellular models that recapitulate both resistant and susceptible forms of the disease are essential to understanding drug resistance and advancing new options for treatment.

OVCAR-3 is a high-grade serous ovarian adenocarcinoma cell line established from a patient refractory to cisplatin, however the cell line remains sensitive to a variety of chemotherapeutic drugs.<sup>2</sup> OVCAR-3 cells demonstrate increased glutathione content3 and express the Wilms Tumor 1 protein (WT1), a marker of advanced serous ovarian carcinoma.<sup>4</sup> OVCAR-3 cells are characterized by migration/invasion ability and are tumorigenic in nude mice.<sup>5</sup> The OVCAR-3 cell line is well-established and one of the most highly-cited model systems for ovarian carcinoma.

#### Source

The OVCAR-3 was established from ascitic fluid from a 60-year-old patient with ovarian cancer refractory to cisplatin treatment.<sup>2</sup>

### **Short Tandem Repeat**

D3S1358: 17, 18 D16S539: 12 TH01: 9 CSF1PO: 11, 12 D21S11: 29, 31.2 Penta D: 12, 13

D18S51: 13 vWA: 17

Penta E: 7, 13 D8S1179: 10, 15

D5S818: 11, 12 TPOX: 8 D13S317: 12 FGA: 21

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.



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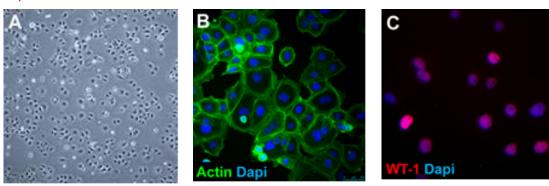
## Quality Control Testing

- Each vial contains  $\geq 1 \times 10^6$  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-3 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-3 cells one day after thaw. (**B**) OVCAR-3 cells express actin (P5282) and (**C**) WT1 (Abcam, ab89901).

### **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-3 Expansion Medium: Cells are thawed and expanded in OVCAR-3 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-3 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-3 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-3 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<sub>2</sub>.

#### Subculturing of Cells

- Do not allow the cells to grow to confluency. OVCAR-3 cells should be passaged at ~80-85% confluence.
  Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of OVCAR-3 cells.
- 2. 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-3 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  $300 \times g$  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-3 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-3 Human Ovarian Carcinoma Cells may be frozen in OVCAR-3 Expansion Medium and 10% DMSO using a Nalgene $^{\rm @}$  slow freeze Mr. Frosty $^{\rm @}$  container.

#### References

- 1. Nat Rev Disease Primers 2016; 2:16061.
- 2. Cancer Res 1997; 57:850-856.
- 3. Proc Natl Acad Sci USA 1992; 89:3070-3074.
- 4. BMC Cancer 2006; 6:90.
- 5. Gynecol Oncol 2015;138(2):372-377.

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