Description

MSLN Knockout OVCAR3 Cell Line is an ovarian cancer OVCAR3 cell line in which human MSLN (Mesothelin) has been genetically removed using CRISPR/Cas9 genome editing. This cell line was generated by electroporation with the Cas9 enzyme (*Streptococcus pyogenes*) and sgRNA targeting human MSLN (Mesothelin).

The cell line has been validated by NGS (next-generation sequencing) and flow cytometry.

Background

Mesothelin (MSLN) is a glycophosphatidylinositol (GPI)-linked cell surface protein that is produced as a ~70 kDa precursor protein and cleaved by Furin protease to generate the ~40 kDa mature form. MSLN is frequently overexpressed in mesothelioma, ovarian, pancreatic, and non-small cell lung (NSCLC) cancers, while its expression in normal tissues is restricted to the mesothelial lining. MSLN is a tumor-associated antigen and has been an attractive target for targeted immunotherapy approaches, including antibodies drug-conjugated (ADCs) and chimeric antigen receptor T cells (CAR-T Cells).

The OVCAR3 cell line was isolated from a high-grade serous ovarian adenocarcinoma patient refractory to treatment with cisplatin. This cell line is highly tumorigenic and presents an abnormal karyotype. OVCAR3 cells are a widely used model of ovarian cancer, particularly for drug resistance studies and hormonal therapy.

Application

- Study the impact of MSLN knockout.
- Use as a negative control during the development of MSLN-directed Biologics and in CAR-T killing assays.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ≥1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

OVCAR3, ovarian adenocarcinoma cell line, adherent.

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 20	BPS Bioscience #84157

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Media Required for Cell Culture

Thaw Medium 20 (BPS Bioscience #84157):

RPMI 1640 medium (ATCC formulation) supplemented with 20% FBS, 1% Penicillin/Streptomycin.

Cell Culture Protocol

Note: OVCAR3 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 20.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 20.

3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium 20, and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Replace media every 2-3 days until cells reach 90% confluency. At first passage and subsequent passages, use Thaw Medium 20.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 20 and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Thaw Medium 20.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:2 to 1:5 once or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 20 and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Data

Figure 1: Genomic sequencing of MSLN in the MSLN Knockout OVCAR3 Cell Line.
Genomic DNA from the MSLN Knockout OVCAR3 cells was isolated and sequenced by NGS sequencing. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) is shown in green, and the Indels (Insertions/Deletions) in the two MSLN alleles are highlighted in red. The reference MSLN genomic DNA is labeled as gDNA.

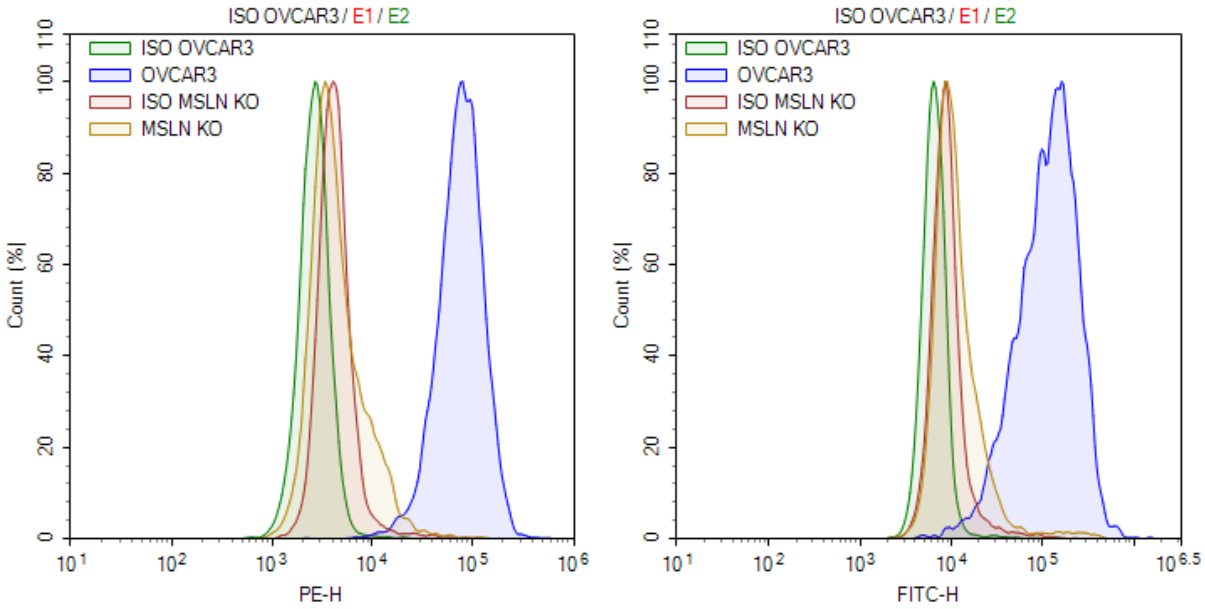

Figure 2. Expression of MSLN in the MSLN Knockout OVCAR3 Cell Line by flow cytometry.
Left: MSLN knockout OVCAR3 cells (orange) and parental OVCAR3 cells (blue) were stained with FluoSite Anti-Mesothelin Antibody, PE-Labeled (#102912) and samples were analyzed by flow cytometry. For isotype controls, MSLN knockout OVCAR3 cells (red) and parental OVCAR3 cells (green) were stained with FluoSite Human IgG1 Kappa Isotype Control Antibody, PE-Labeled (#103024).
Right: MSLN knockout OVCAR3 cells (orange) and parental OVCAR3 cells (blue) were stained with 1 µg of Amatuximab antibody (#84162), followed by Alexa Fluor 488–conjugated AffiniPure Goat Anti-Human IgG (H+L) secondary antibody (#84163). Samples were subsequently analyzed by flow cytometry. For isotype controls, MSLN knockout OVCAR3 cells (red) and parental OVCAR3 cells (green) were stained with Human IgG1 kappa, Isotype Control antibody (#83956).

Data shown is representative.

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Notes
The CRISPR/CAS9 technology is covered under numerous patents, including U.S. Patent Nos. 8,697,359 and 8,771,945, as well as corresponding foreign patents applications, and patent rights.

Version 041326