Description

TROP2 Knockout HeLa Cell Line is a HeLa cell line in which human TROP2 (TACSTD2) has been genetically removed using CRISPR/Cas9 genome editing with a lentivirus encoding and stably expressing CRISPR/Cas9 gene and sgRNA (single guide RNA) targeting human TROP2.

This cell line has been validated by genomic sequencing and flow cytometry.

Background

TROP2 (Trophoblast cell-surface antigen 2), also referred to as tumor associated calcium signal transducer 2 (TACSTD2), GA733-1 or M1S1, is a cell surface glycoprotein that is highly expressed in a variety of solid cancers yet has low expression in normal cells. Through a variety of signaling pathways, such as the MAPK (mitogen-activated protein kinase)/PI3K (phosphoinositide 3-kinase)/AKT (protein kinase B) pathway TROP2 regulates cancer growth and metastasis. Overexpression of this protein is associated with a poor prognosis in cancers such as breast, gastric and ovarian cancer. In other cases, such as NSCLC (non-small cell lung cancer) lower expression seems to link to the malignancy. TROP2 is a favorable target for antibody drug conjugates (ADC) and immunotherapy. Studies with Sacituzumab govitecan, an ADC composed of an anti-TROP2 humanized monoclonal antibody and SN-38 (topoisomerase I inhibitor) have shown promising results in cell models.

Application

- Use as a negative control when testing TROP2 inhibitors or antibodies in HeLa cells.
- Study the phenotype resulting from TROP2 knockout.

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

HeLa, Human epithelial cells, 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 6	BPS Bioscience #60183

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BPS Bioscience ● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics.
Cells should be grown at 37˚C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 6 (BPS Bioscience #60183)
DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Note: Although the cells do not need to be grown in puromycin to maintain TROP2 knockout, the cells are puromycin resistant.

Cell Culture Protocol

Note: HeLa 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 6.

   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 6.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 6 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 6.

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.

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2. Once the cells have detached, add Thaw Medium 6 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 6.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:2 to 1:10 weekly 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 6 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

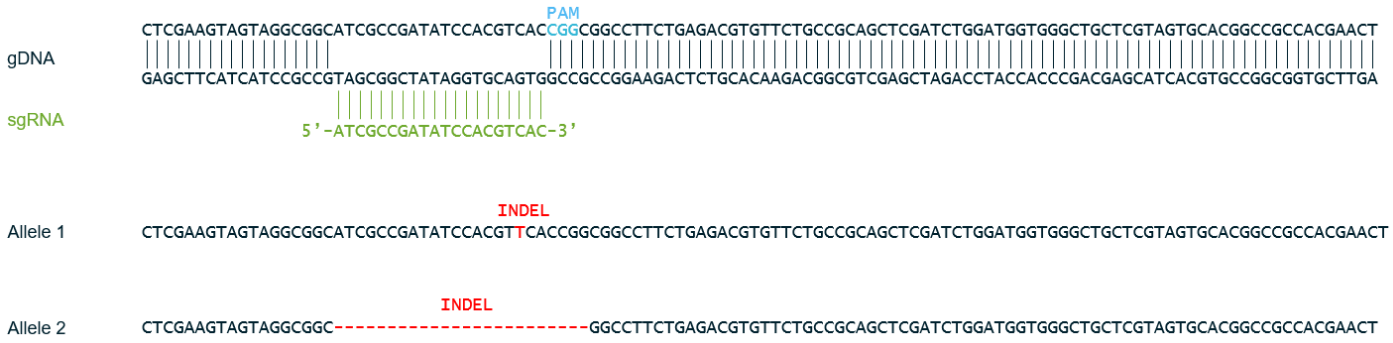

Figure 1: Genomic sequencing of TROP2 in the TROP2 Knockout HeLa Cell Line.
Genomic DNA from the TROP2 Knockout HeLa cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) is shown in green, and the Indels (Insertions/Deletions) in the two TROP2 alleles are highlighted in red. The TROP2 genomic DNA is labeled as gDNA.

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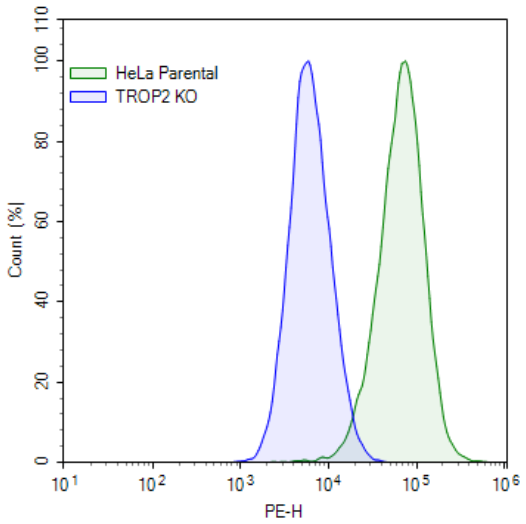

Figure 2: TROP2 expression in TROP2 Knockout HeLa Cell Line by flow cytometry.
Parental HeLa cells (green) and TROP2 Knockout HeLa cells (blue) were stained with a PE-labeled anti-human TACSTD2 (TROP2) Antibody (BioLegend #363803) and analyzed by flow cytometry. The Y-axis is the % cell number. The X-axis is the intensity of PE.

Data shown is representative.

Troubleshooting

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

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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.

Related Products

Products	*Catalog #*	*Size*
Trop2 Lentivirus	78710	500 µl x 2
Trop2 Lentivirus (Macaca fascicularis/Cynomolgus)	78776	500 µl x 2
TROP2 – CHO-K1 Recombinant Cell Line	78099	2 vials
Trop2, Fc Fusion (IgG1), Avi-Tag Recombinant	101344	100 µg/1 mg
Trop2 (88-274), Fc Fusion (IgG1), Avi-Tag Recombinant	101346	100 µg/1 mg
Trop2 (88-274), Fc Fusion (IgG1), Avi-Tag, Biotin-Labeled Recombinant	101347	25 µg/100 µg

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