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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

CD38 Knockout A549 Cell Line is a A549 lung cancer cell line in which human CD38 (Cluster of Differentiation 38) has been genetically removed using CRISPR/Cas9 genome editing with a lentivirus encoding the CRISPR/Cas9 gene and sgRNA (single guide RNA) targeting human CD38.

Background

CD38 (Cluster of Differentiation 38, Cyclic ADP-Ribose Hydrolase 1, or ADPRC1) is a glycoprotein and ectoenzyme playing an important role in regulating intracellular calcium. CD38 is a highly attractive target antigen for immunotherapy because it is highly expressed on multiple myeloma (MM) cells, and at relatively low levels on normal lymphoid and myeloid cells. Expression of CD38 has also been associated with HIV infection, leukemia, and type II diabetes mellitus (T2D). In 2015, the FDA approved daratumumab (Darzalex), a breakthrough therapy monoclonal antibody targeting CD38, for the treatment of MM. CD38 has become an interesting target for the development of CAR (chimeric receptor antigen)-T cells, which are T cell product engineered by retroviral transduction to express a fully human CD38-specific CAR. The level of upregulation of CD38 in lung cancer patients correlates with patient survival, with its enzymatic activity leading to increased cell migration, proliferation and tumor progression. Future development and studies will open new therapeutic avenues for CD38-related diseases, such as lung cancer.

Application

- Use as a negative control when testing CD38-directed therapies in A549 cells.
- Study the phenotype resulting from CD38 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

A549 is a human lung alveolar vessel carcinoma cell line. Adherent epithelial cells.

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

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.

Note: Thaw Media do *not* contain selective antibiotics.
Cells should be grown at 37℃ 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 CD38 knockout, the cells are puromycin resistant.

Cell Thawing

Note: Note: A549 cells are derived from human material and thus the use of adequate safety precautions is recommended.

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.

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 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 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

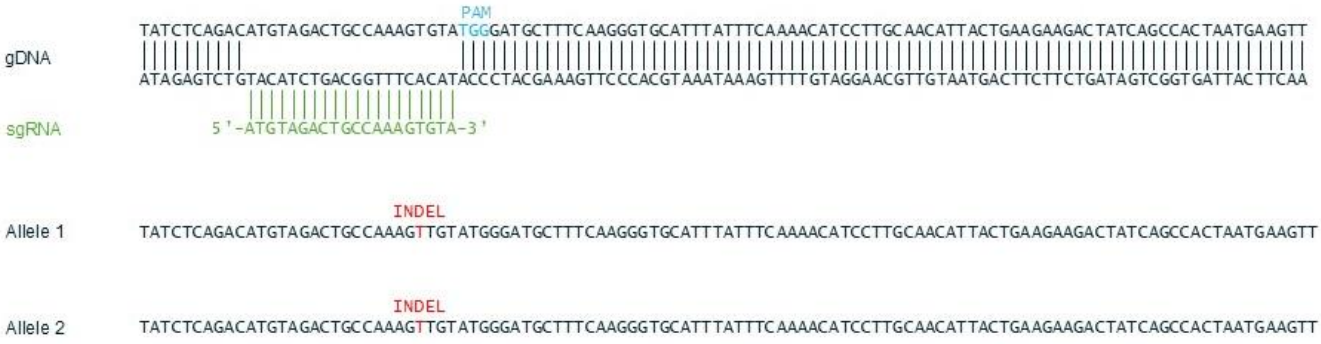

Figure 1: Genomic sequencing of CD38 in the CD38 Knockout A549 Cell Line.
Genomic DNA from CD38 Knockout A549 cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) is shown in green, and the Indels (Insertions/Deletions) in the two CD38 alleles are highlighted in red. The CD38 genomic DNA is labeled as gDNA.

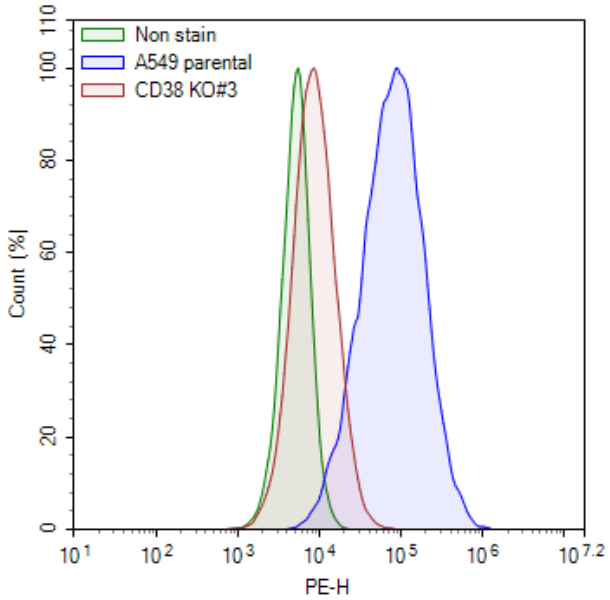

Figure 2. Expression of CD38 in the CD38 Knockout A549 Cell Line.
CD38 Knockout A549 cells (red) or parental A549 cells (blue) were stained with PE anti-human CD38 Antibody (BioLegend #303506) and analyzed by flow cytometry. Unstained parental A549 cells are shown in green. Y-axis is the % cell number. X-axis is the intensity of PE.

Data 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.

References
Cui Q., *et al.*, 2021 *J Hematol Oncol.* 14(1):82.
Gao L., *et al.*, 2021 *Cell Death Dis.* 12(7):680.

Related Products

Products	*Catalog #*	*Size*
CD38 CHO Recombinant Cell Line (High, Medium or Low Expression)	79615	2 vials
CD38/ BCMA/ Firefly CHO Recombinant Cell Line	78148	2 vials
CD38/ CD19/ Firefly Luciferase CHO Recombinant Cell Line	78149	2 vials
Anti-CD38 Antibody	79120	25 µg/ 100 µg
CD38 Inhibitor Screening Assay Kit (Cyclase Activity)	71275	96 reactions
CD38 Inhibitor Screening Assay Kit (Hydrolase Activity)	79672	384 reactions

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