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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

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Description

Nectin-4 Knockout MCF7 Cell Line is an MCF7 breast cancer cell line in which human Nectin-4 has been genetically removed using CRISPR/Cas9 genome editing with a lentivirus encoding CRISPR/Cas9 gene and sgRNA (single guide RNA) targeting human Nectin-4.

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

Background

Nectin-4 is part of the Nectin family and plays a crucial role in cell adhesion. Nectin-4 contains two immunoglobulin-like C2-type domains and one Ig-like V-type domain. In contrast to other Nectins, which are found extensively in adult tissues, Nectin-4 is abundant during fetal development but declines in adult life. Its expression, however, returns specifically in lung, breast, pancreas and ovarian cancers. It was shown that Nectin-4 can modulate the expression of epithelial–mesenchymal transition-related proteins via the PI3K (phosphoinositide 3-kinase)/AKT (protein B kinase) pathway. In addition, a recent study demonstrated that Nectin-4 is a cancer-specific TIGIT ligand, and its expression is also associated with poor prognostic features, suggesting it could be an efficient target for cancer immunotherapy. In 2019, the FDA approved Enfortumab vedotin-ejfv, which is a Nectin-4-directed antibody drug conjugate (ADC) used in treating metastatic urothelial cancer.

Application

- Study phenotypes resulting from Nectin-4 knockout.
- Use as negative control in the development of ADC or other biologics targeting Nectin-4.

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

MCF7 human breast mammary gland 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 1	BPS Bioscience #60187
Insulin Solution from Bovine Pancreas	Sigma-Aldrich #I0516

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°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

Complete Thaw Medium 1:

Thaw Medium 1 (BPS Bioscience #60187) + 10 µg/ml Insulin (Sigma-Aldrich #I0516): MEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin, 1% Non-Essential amino acids, and 1 mM Na pyruvate + 10 µg/ml Insulin (Sigma-Aldrich #I0516).

Note: the final concentration of 10 µg/ml Insulin (Sigma-Aldrich #I0516) will need to be added to Thaw Medium 1 for cell culture.

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 Complete Thaw Medium 1.

   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 Complete Thaw Medium 1.

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 Complete Thaw Medium 1 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 Complete Thaw Medium 1.

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 Complete Thaw Medium 1 and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Complete Thaw Medium 1.

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 Complete Thaw Medium 1 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 Nectin-4 in the Nectin-4 Knockout MCF7 Cell Line.
Genomic DNA from the Nectin-4 Knockout MCF7 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 Nectin-4 alleles are highlighted in red. The Nectin-4 genomic DNA is labeled as gDNA.

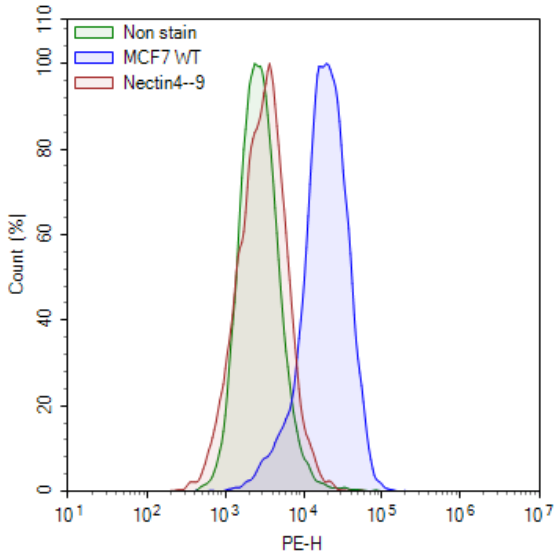

Figure 2: Expression of Nectin-4 in Nectin-4 Knockout MCF7 Cell Line by flow cytometry. Cells were stained with anti-human Nectin-4 Antibody, REAfinity™ (Miltenyi Biotech #130-116-027) and analyzed by flow cytometry. Parental MCF7 cells are shown in blue, unstained parental MCF7 cells are shown in green, and the Nectin-4 Knockout MCF7 cells are shown in red. The y axis shows the % of cells, while the x-axis represents the fluorophore intensity.

Results are representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

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*
Nectin-4 Lentivirus	78712	500 µl x 2
Nectin4- CHO K1 Recombinant Cell Line (High, Medium, or Low Expression)	78097	2 vials
Nectin4, His-Avi-Tag HiP™ Recombinant	100674	100 µg/1 mg
Nectin4, His-Avi-Tag, Biotin-labeled Recombinant	100675	25 µg/50 µg

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