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Description

MUC1 CHO Cell Line is a CHO-K1 cell line expressing MUC1 variant 2 (also known as Mucin-1 or CD227; NM_001018016.3) under the control of a cytomegalovirus (CMV) promoter. This cell line was generated by lipid-mediated transfection followed by geneticin selection and limited dilution. Individual clones were screened for MUC1 expression levels by flow cytometry, and a clone was selected to generate this cell line.

Background

MUC1 (mucin-1) is a transmembrane protein involved in cell adhesion in epithelial cells as well as helping to provide a layer of protection against bacterial and enzyme attack. It is broadly expressed in human epithelial tissues however, MUC1 is also an oncogene that is overexpressed in ovarian, breast, gastric and some non-epithelial tumors. Aberrant expression of MUC1 upregulates cell growth, metastasis, proliferation and other functions in cancer and correlates with poor prognosis and survival time in patients. The extracellular region of MUC1 is highly glycosylated (*O*- and *N*-linked carbohydrates and sialic acid) and contains a polymorphic region of variable number of tandem repeats (VNTR) region as well as a SEA (sperm protein, enterokinase and agrin) domain. These glycosylated regions help form a physical barrier towards exogenous pathogens and participate in defense against pathogens. The SEA domain contains a cleavage site which has been indicated to also play a role in defense against microorganisms. This cell line expresses isoform 2 which is highly expressed in tumor tissues. While the cytoplasmic tail region and associated signaling functions from MUC1 isoform 1 are preserved, isoform 2 has a truncated extracellular domain that lacks the VNTR region and a 15 amino-acid deletion within the SEA domain. As a result, it does not undergo cleavage. Due to its cell surface expression in a variety of cancers and correlation with patient prognosis, MUC1 is a prospective therapeutic target for treatments including CAR-T Cell Therapy, MUC1-activated T cell treatment and MUC1 vaccination.

Applications

- Screen therapeutic antibodies and ADCs targeting MUC1
- MUC1 vaccination with or without co-treatment targeting MUC1

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

CHO-K1 cells, Chinese Hamster Ovary, epithelial-like 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 3	BPS Bioscience #60186
Growth Medium 3D	BPS Bioscience #79539

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. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest. 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 3 (BPS Bioscience #60186):

F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 3D (BPS Bioscience #79539):

F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml of Geneticin.

Cell Culture Protocol

Cell Thawing

1. Thaw the vial of frozen cells for 30-60 seconds in a 37°C water bath. As soon as the cells are thawed, quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 3.

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 $400 \times g$ for 2 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 3.
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 3 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 3D.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without $\text{Ca}^{2+}/\text{Mg}^{2+}$, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 3D and transfer to a tube.
3. Spin down cells at $400 \times g$ for 2 minutes, remove the medium and resuspend the cells in Growth Medium 3D.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:6 to 1:12 once or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without $\text{Ca}^{2+}/\text{Mg}^{2+}$, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 3D and count the cells.
3. Spin down the cells at $400 \times g$ for 2 minutes, remove the medium and resuspend the cells in 4°C Freezing Medium (BPS Bioscience #79796) at $\sim 2 \times 10^6$ cells/ml.
4. Dispense 1 ml of cell suspension into each cryogenic vial.
5. Place the vials in an insulated container for slow cooling and store at -80°C overnight.
6. 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.

A. Validation Data

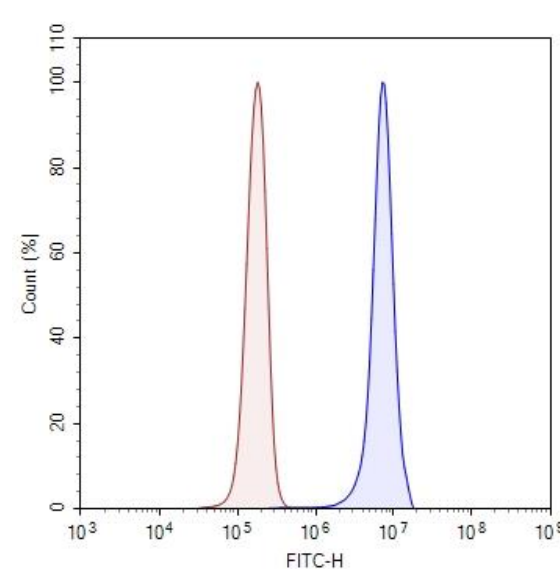


Figure 1: Flow cytometry analysis of MUC1 expression in MUC1 CHO Cell Line.

Cytometry was conducted on MUC1 CHO cell line (blue) and control parental CHO-K1 cells (red) after intracellular staining at room temperature using the following conditions: For each test, 0.5 million cells were incubated in Fixation Buffer (BioLegend #420801) mixed at an equal volume ratio with permeabilization buffer (BioLegend #421002) for 20 minutes. The cells were washed with permeabilization buffer and then blocked with Human TruStain (BioLegend #422301) for 10 minutes. MUC1 antibody (Thermo Fisher #MA5-11202) was added to the incubation mixture at a total volume of 100 μl and the cells were incubated for 30 more minutes. They were then washed and incubated with anti-IgG (Armenian Hamster – BioLegend #405502) in permeabilization buffer

for 20 minutes in the dark. After washing, the cells, they were resuspended in permeabilization buffer. The cells were analyzed by flow cytometry with the y-axis representing the % cell number and X-axis indicating FITC intensity.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Sequence

Human MUC1 sequence (accession number NM_001018016.3)

MTPGTQSPFFLLLLLTVLTATTAPKPATVVTGSGHASSTPGGEKETSATQRSSVPSSTEKNAFNSSLEDPSTDYYQELQRDISEMFL
QIYKQGGFLGLSNIKFRPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISDVSVDVPPFSAQSGAGVPGWGIAL
VLVCVLVALAIVYLIALAVCQCRRKNYGQLDIFPARDTYHPMSEYPTYHTHGRYVPPSSTDRSPYEKVSAGNGGSSLSYTNPAVAA
TSANL

References

Nivet C., et al., 2024 *Biomedicines* 12(1), 139.
Kumar S., et al., 2017 *Carcinogenesis* 38(7): 671-679.
Chen W., et al. 2021 *Int J Mol Sci.* 22(12): 6567.

License Disclosure

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Troubleshooting Guide

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

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
MUC16 (CA125), variant 4 (region 13785-14507) CHO Cell Line	78848	2 vials
p53 Luciferase Reporter Lentivirus	78666	500 µl x 2
p53 Luciferase Reporter HCT116 Cell Line	78681	2 vials

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