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Description

Firefly Luciferase GPC3 CHO Cell Line is a CHO-K1 cell line that expresses the firefly luciferase reporter and constitutively expresses human GPC3 (Glypican 3) (Genbank# NM_004484.2).

This cell line has been validated by flow cytometry and luciferase activity.

Background

GPC3, also known as Glypican-3 and OCI5, belongs to the glypican family and is highly expressed in the lungs, liver and kidneys. Its function is tissue dependent and can either promote or suppress tumorigenesis. Being a heparin sulfate proteoglycan, it is overexpressed in neoplasms including malignant melanoma, hepatocellular carcinoma (HCC), and testicular yolk sac tumors and plays a significant role in cell growth and differentiation. Due to its highly restricted expression in normal tissues and high prevalence in many solid tumors, GPC3 has become an attractive target for antibody drug conjugates (ADC), immune engagers, and for chimeric antigen receptor (CAR) T cell therapy.

Application

- Use as target cells in CAR-T or NK co-culture killing assays.
- *In vitro* and *in vivo* bioluminescence imaging.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains $\geq 1 \times 10^6$ 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 3C	BPS Bioscience #79537

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% CO₂. 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 (Kaighn's Modification of Ham's F-12 Medium) supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 3C (BPS Bioscience #79537):

F-12K Medium (Kaighn's Modification of Ham's F-12 Medium) supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 500 µg/ml of Hygromycin B and 5 µg/ml of Puromycin Dihydrochloride.

Cell Culture Protocol

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 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 300 x g for 5 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 and incubate at 37°C in a 5% CO₂ incubator.
4. After 48-72 hours of culture, check for cell viability, change to fresh Thaw Medium 3, and continue growing in a 5% CO₂ incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they reach 100% confluency. Switch to Growth Medium 3C for passage.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca²⁺/Mg²⁺, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA following volumes recommended for the cell vessel being used.
2. Once the cells have detached, add Growth Medium 3C and transfer to a tube.
3. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 3C.

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

Cell Freezing

- 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 following volumes recommended for the cell vessel being used.
- Once the cells have detached, add Growth Medium 3C and transfer to a tube.
- Spin down cells at $300 \times g$ for 5 minutes.
- Remove the medium and resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of $\sim 2 \times 10^6$ cells/ml.
- 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.
- 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

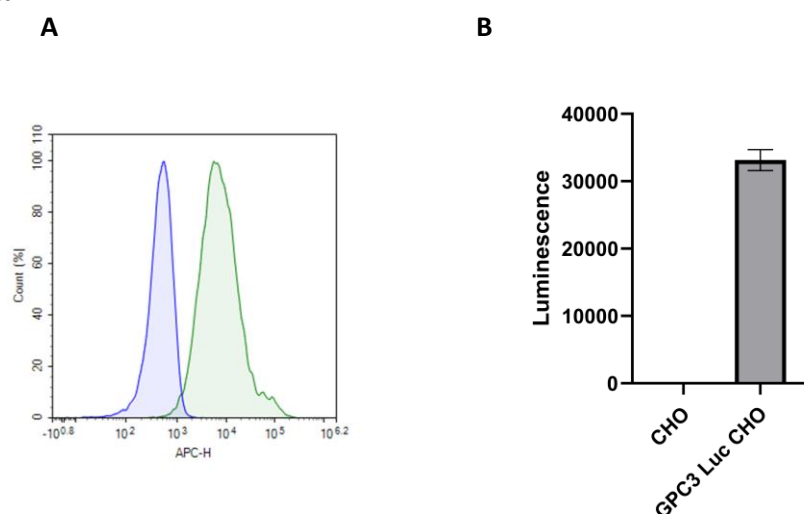


Figure 1. Flow cytometry to assess GPC3 expression and luciferase activity measurement in Firefly Luciferase GPC3 CHO Cell Line.

A. Firefly Luciferase GPC3 CHO cells (green) or control CHO K1 cells (blue), were labelled with Human Glypican 3 APC-conjugated Antibody (R&D Systems #FAB2119A) and analyzed by flow cytometry. The y axis represents the % of cells, while the x axis indicates the fluorophore intensity.

B. Luciferase activity in Firefly Luciferase GPC3 CHO cells was measured using One Step™ Luciferase Assay System (#60690).

Data is representative.

Sequence

Human GPC3 (Glypican 3) sequence (accession number NM_004484.2)

MAGTVRTACLVVAMLLSLDFPGQAQPPPPPDATCHQVRSFFQRLQPGLKWVPETPVPGSDLQVCLPKGPTCCSRKMEEKYQL
 TARLNMEQLLQSASMEKFLIIQNAAVFQEAFAFEIVVRHAKNYTNAMFKNNYPSLTPQAFFVGEFFTDVSLYLGSINVDMMVN
 ELFDSLFPVIYTQLMNPGLPDSALDINECLRGARRDLKVFGNFPKLIMTQVSKSLQVTRIFLQALNLGIEVINTTDHLKFSKDCGRML
 TRMWYCSYCQGLMMVKPCGGYCNVVMQGC MAGVVEIDKYWREYILSLEELVNGMYRIYDMENVLLGLFSTIHDSIQYVQKNA
 GKLTITIGKLCAHSQQRQYRSAYYPEDLFIDKKVLKVAHVEHEETLSSRRRELIQKLKSFISFYSALPGYICSHSPAENDTLCWNGQ
 ELVERYSQKAARNGMKNQFNLHELKMKGPEPVVSQIIDKLKHINQLLRTMSMPKGRVLDKNLDEEGFESGDCGDDDECIGGS
 GDGMIVKNQLRFLAELAYDLDDVDDAPGNSQQATPKDNEISTFHNLGNVHSPLKLLTSMAISVVCFFFLVH

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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 email visit <https://bpsbioscience.com/contact>.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Anti-GPC3 CAR-T Cells	82492	1 vial
Anti-GPC3 CAR Lentivirus (Clone GC33-CD28TM-41BB-CD3ζ)	82494	50 µl
GPC3 – CHO-K1 Recombinant Cell Line	78100	2 vials
GPC3 Lentivirus	78711	500 µl x 2

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