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Produktinformation

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

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

Anti-GPC3 CAR-T Cells are produced by high-titer lentiviral transduction of human primary $CD4^+$ and $CD8^+$ T cells using Anti-GPC3 CAR Lentivirus (Clone GC33-CD28TM-41BB-CD3ζ) (BPS Bioscience #82494). These ready-to-use CAR-T cells express an anti-GPC3 (glypican-3) CAR (chimeric antigen receptor) consisting of the ScFv portion of anti-GPC3 (clone GC33) linked to a 2nd generation CAR, containing IgG1 short hinge and CD28 transmembrane domains, 4-1BB and CD3ζ signaling domains (Figure 1).

These CAR-T cells have been validated using flow cytometry (to determine CAR expression) and co-culture cytotoxicity assays.

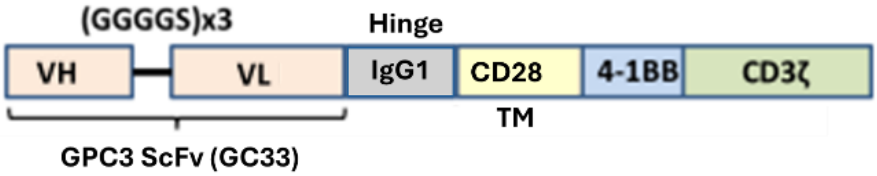

Figure 1. Construct diagram showing components of the Anti-GPC3 CAR expressed in Anti-GPC3 CAR-T Cells.

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, 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 chimeric antigen receptor (CAR) T cell therapy.

Application

- Use as positive control in the development of anti-GPC3 CAR-T cells.
- Screen modulators of anti-GPC3 CAR-T cytotoxicity.
- Design and optimize co-culture cytotoxicity assays for anti-GPC3 specific CAR-T cell evaluation.

Biosafety

The anti-GPC3 CAR-T cells are produced with the third generation SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal and are not present in the lentivirus particle.

Materials Provided

Components	Format
One vial of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of CryoStor® CS10 (Stemcell Technologies #100-1061)

Mycoplasma Testing

The cells have been screened to confirm the absence of Mycoplasma species.

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.

Materials Required but Not Supplied

These materials are not supplied with the CAR-T cells but are necessary for cell culture and for the cellular assays described below. BPS Bioscience's reagents are validated and optimized for use with these cells and are highly recommended for best results.

Name	Ordering Information
TCellM™	BPS Bioscience #78753
Human Interleukin-2 Recombinant	BPS Bioscience #90184
GPC3, Avi-His-Tagged, Biotin-Labeled Recombinant	BPS Bioscience #100072
PE anti-Biotin Antibody	BioLegend # 409003
Firefly Luciferase A549 Cell Line	BPS Bioscience #82495
Firefly Luciferase HepG2 Cell Line	BPS Bioscience #82490
Thaw Medium 1	BPS Bioscience #60187
Thaw Medium 6	BPS Bioscience #60183
Untransduced T Cells (Negative Control for CAR-T cells)	BPS Bioscience #78170
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Recommended CAR-T Cell Medium: TCellM™ (#78753) supplemented with 10 ng/ml of Interleukin-2 (#90184).

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 CAR-T Cell Medium.

   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 CAR-T Cell Medium.

3. Transfer the resuspended cells to a T25 flask.

Cell culture

1. Centrifuge the cells gently at 300 *x g* for 5 minutes.

2. Resuspend in fresh CAR-T Cell Medium.

3. Continue to culture the cells at 37°C with 5% CO_2.

4. Do not allow the cell density to exceed 2.0 x 10^6 cells/ml. Transfer the cells in larger culture vessels and add fresh medium when the density reaches 2.0 x 10^6 cells/ml.

Perform the cytotoxicity assay as soon as possible to avoid exhaustion. Anti-GPC3 CAR-T cells may stop proliferation after ~one week in culture. Cells can be activated again for expansion. It is not recommended to freeze the cells again once they have been activated and expanded.

Validation Data

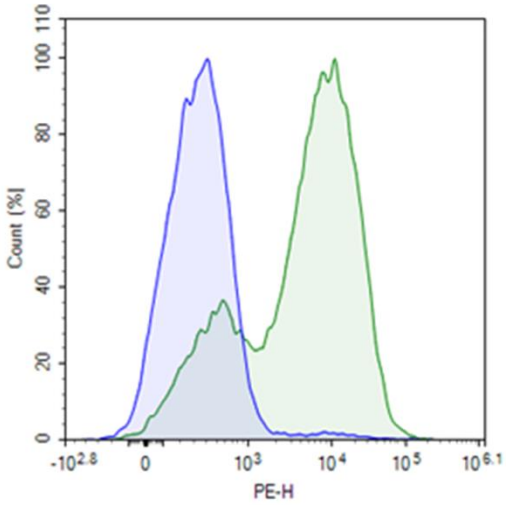

Figure 2. Expression of anti-GPC3 CAR in Anti-GPC3 CAR-T Cells analyzed by flow cytometry. Anti-GPC3 CAR-T cells (green) and Unstransduced T cells (#78170) (blue) were thawed for 24 hours and stained with GPC3, Avi-His-Tagged, Biotin-Labeled Recombinant (#100072) and PE anti-Biotin Antibody (BioLegend #409003). Anti-GPC3 expression was analyzed by flow cytometry. The y axis represents the % of cells, while the x axis indicates PE-intensity.

Functional Validation

Cytotoxicity assay of Anti-GPC3 CAR-T Cells using Firefly Luciferase A549 Cell Line and Firefly Luciferase HepG2 Cell Line as the target cells

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The assay should include “Background Control”, “No T Cell Control” and “Test” conditions.
- The following experiments are an example of co-culture assays where Firefly Luciferase A549 Cell Line (low GPC3 expression level) and Firefly Luciferase HepG2 Cell Line (#82490) (high GPC3 expression level) are used to evaluate the cytotoxicity of Anti-GPC3 CAR-T Cells (#82492).
- We recommend the use of Untransduced T Cells (Negative Control for CAR-T cells) (#78170) as control.

T Cell Medium: TCellM™ (#78753) supplemented with 10 ng/ml of Interleukin-2 (#90184).

Day 0

1. Thaw frozen Anti-GPC3 CAR-T cells and control cells in T Cell Medium.

2. Incubate at 37°C for 24 hours.

Day 1

1. Seed Firefly Luciferase A549 cells and Firefly Luciferase HepG2 cells in 50 µl of Thaw Medium 6 and Thaw Medium 1, respectively, at 500 cells/well in a 96-well white, clear bottom tissue culture plate. Leave a few empty wells as "Background Control".

2. Centrifuge T cells gently and resuspend in fresh T Cell Medium at the appropriate cell density to reach the desired effector:target (E:T) cell ratio (50 µl/well).

3. Carefully pipet 50 µl of T cells into each well at the desired effector:target (E:T) cell ratio to the "Test" wells.

4. Add 50 µl of fresh T Cell Medium to the "No T Cell Control" wells.

5. Add 100 µl of fresh T Cell Medium to the "Background Control" wells.

6. Incubate at 37°C for 24 hours.

   Note: No overnight attachment is needed for the adherent target cells. T cells can be added into the wells 1-2 hours after the target cells were seeded.

Day 2

1. Add 50 µl of ONE-Step™ Luciferase assay reagent to each well.

2. Incubate at Room Temperature (RT) for ~15 to 30 minutes before measuring luminescence using a luminometer.

   Data Analysis: the average background luminescence was subtracted from the luminescence reading of all wells. The luciferase activity of Firefly Luciferase A549 cells and Firefly Luciferase HepG2 cells was set as 100%. The % Luminescence was calculated as background-subtracted luminescence of co-culture wells divided by background-subtracted luminescence of the "No T Cell Control" wells (Firefly Luciferase A549 cells and Firefly Luciferase HepG2 cells only).

$$\%\ Lum = \frac{Lum\ coculture - background}{Lum\ control - background} * 100$$

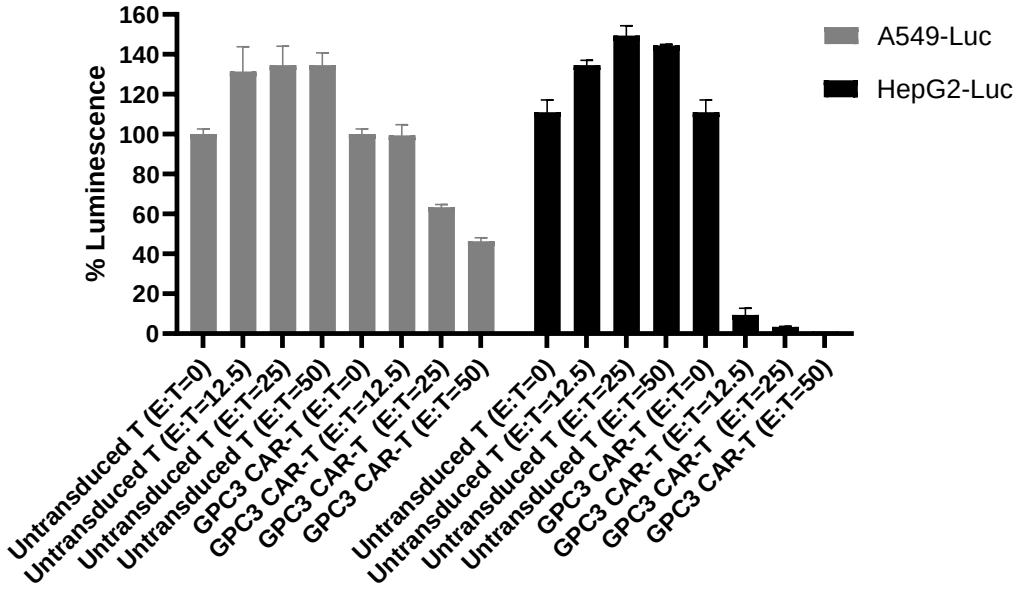

Figure 3. Luciferase-based cytotoxicity assay of Anti-GPC3 CAR-T Cells using Firefly Luciferase A549 Cell Line and Firefly Luciferase HepG2 Cell Line as the target cells.
Anti-GPC3 CAR-T cells (#82494) (effector) were thawed and were co-cultured with Firefly Luciferase A549 cells (#82495) or Firefly Luciferase HepG2 cells (#82490) as the target cells (target) for 24 hours at the indicated E:T ratios. The lysis of the target cells was determined by measuring luciferase activity with ONE-Step™ Assay System (#60690). The assay was performed in parallel with untransduced T cells as a negative control.

Data is representative.

References
Li W., *et al.*, 2017 Hum Gene Ther. 28(5):437-448.

Warnings
Donors have been screened and determined negative for:
- Hepatitis B (anti-HBc EIA, HBsAg EIA)
- Hepatitis C (anti-HCV EIA)
- Human Immunodeficiency Virus (HIV-1/HIV-2 plus O)
- Human T-Lymphotropic Virus (HTLV-I/II)
- HIV-1/HCV/HBV
- West Nile Virus
- Trypanasoma cruzi

Note: *Testing cannot guarantee that any sample is completely virus-free. These cells should be treated as potentially infectious and appropriate Biological Safety Level 2 (BSL-2) precautions should be used.*

Troubleshooting Guide

Visit Cell Line FAQs for more information. For lot-specific information and all other questions, please email visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
Anti-CD19 CAR-T Cells	78171	1 vial
Firefly Luciferase Raji Cell Line	78622	2 vials
Firefly Luciferase K562 Cell Line	78621	2 vials
Firefly Luciferase - CHO Recombinant Cell Line	79725	2 vials
CD19 / Firefly Luciferase - CHO Recombinant Cell Line	79714	2 vials
Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ)	78600	50 µl

Version 060525