SZABO SCANDIC

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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
The anti-CD19 CAR lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to be transduced into almost all types of mammalian cells, including primary and non-dividing cells. These viruses constitutively express the ScFv portion of anti-CD19 (clone FMC63) linked to 2nd generation CAR (Chimeric Antigen Receptor) containing CD8 hinge, 4-1BB, and CD3ζ signaling domains (Figure 1).

Application
Ideal as a positive control for anti-CD19 CAR evaluation in T cells; useful for transduction optimization.

Formulation
The lentiviruses were produced from HEK293T cells, concentrated, and resuspended in DMEM.

Titer
One vial (50 µl) of anti-CD19 CAR at a titer ≥ 10^8 TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

Storage
Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the virus at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety
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. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

Materials Required but Not Supplied
These materials are not supplied with this lentivirus but are necessary to follow the designed protocol. BPS Bioscience media, reagents, and luciferase assay systems are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
PBMC, Frozen	BPS Bioscience, #79059
Human Interleukin-2	BPS Bioscience, #90184
EasySep™ Human CD4+ T Cell Isolation Kit	Stemcell technologies, #17952
EasySep™ Human CD8+ T Cell Isolation Kit	Stemcell technologies, #17953
Human CD3/CD28/CD2 T Cell Activator	Stemcell technologies, #10970
PE-Labeled Anti-FMC63 scFv Monoclonal	Acrobiosystems, # FM3-HPY53-25tests
IFN-γ (Human) Colorimetric ELISA Detection Kit	BPS Bioscience, #79777
CD19 / Firefly Luciferase - CHO Recombinant Cell Line	BPS Bioscience, #79714
Firefly Luciferase - CHO Recombinant Cell Line	BPS Bioscience, #79725
ONE-Step™ Luciferase Assay System	BPS Bioscience, #60690

Media Formulation

Recommended CD4+CD8+ T Cell Medium:
StemSpan SFEM (Stemcell Technologies, #09650) supplemented with 10% heat-inactivated FBS (Life Technologies, #10082147), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01), plus 10 ng/ml IL-2 (BPS Bioscience, #90184).

Assay Protocol:

The following protocols are a general guideline for transducing CD4+CD8+ primary T cells using anti-CD19 CAR Lentivirus. The transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirements.

1. Day 0: Isolate CD4+ T cells and CD8+ T cells from equal amounts of previously frozen human PBMC by negative selection, according to manufacturer's instruction. Mix the CD4+ T cells and CD8+ T cells after isolation and culture the cells in recommended T cell medium at 1 x 10^6 cells/ml density. Incubate cells at 37°C with 5% CO_2 overnight.

2. Day 1: Add T cell activation reagents. Incubate the plate at 37°C with 5% CO_2 for 24 - 48 hours.

3. Day 2: Thaw anti-CD19 CAR lentivirus and keep it on ice.

   Centrifuge T cells and resuspend them in fresh T cell medium at 0.1 - 0.2 x 10^6 cells/ml; add 5 µg/ml polybrene into the cells;

   Spinoculation:
   1) Pipette 100 µl of T cells (~10,000-20,000) into each 1.5 ml Eppendorf tube.
   2) Calculate the amount of virus needed at desired MOI and add the virus to the T cells. We recommend titrating the MOI, starting from 20. Incubate in the hood at room temperature for 10 minutes; spin the cells/virus at 800 x g for 2 hours at 32°C.
   3) Add 900 µl of fresh T cell medium into each well of a 24-well plate; resuspend the cells/virus from spinoculation and add them into the 24-well plate. It is not necessary to remove the virus. Incubate the cells at 37°C with 5% CO_2 for ~72 hours.

4. Day 5: Check the expression of the anti-CD19 CAR by flow cytometry using PE-Labeled anti-FMC63 scFv antibody. Continue expanding the transduced T cells using recommended T cell medium.

   Once the transduced cells have proliferated sufficiently to reach the desired cell number required for your experiments, use the cells as soon as possible to minimize cellular exhaustion. In our experience at BPS Bioscience, by 11 days post-transduction, the T cells have expanded >1000 fold using our recommended T cell medium.

5. Day 12: To validate anti-CD19 CAR-T activity, seed the target cells CD19-Luciferase CHO cells (BPS Bioscience, #79714) and Luciferase CHO cells (as negative control; BPS Bioscience, #79725) in 50 µl CHO growth medium (BPS Bioscience, #60186) at 500 cells/well in a 96-well white, clear bottom tissue culture plate. Incubate the plates overnight and let the CHO cells attach.

6. Day 13: Spin down T cells and resuspend in fresh T cell growth medium. Add T cells into each well at desired effector:target (E:T) cell ratio in 50 µl of volume. We recommend titrating the E:T ratio starting from 10:1. For "no T cells" control, add 50 µl of fresh T cell medium. The total volume of each well is 100 µl. Incubate the plates at 37°C for 24 hours.

7. Day 14: Pipette up and down gently 3 to 4 times in each well using a multichannel pipet. Transfer the medium/nonattached cells into another plate.

   For the CAR-T/CHO cells coculture plate, proceed with **Luciferase assay**; for the collected medium/nonattached cells, proceed with **IFNγ analysis.**

   Luciferase assay: Prepare the ONE-Step™ Luciferase reagent (BPS Bioscience, #60690) per recommended protocol. Add 50 µl of ONE-Step™ Luciferase assay reagent per well. Add 50 µl Luciferase assay reagent into several empty wells for determine the background luminescence. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

   Data Analysis: subtract the average background luminescence from the luminescence reading of all wells. The luciferase activity of Luciferase CHO cells or CD19 Luciferase CHO cells are set as 100%. The % Luminescence=background subtracted luminescence of coculture well/background subtracted luminescence from the "no T cells" well (Luciferase CHO or CD19 Luciferase CHO cells only).

Note: the luciferase activity from CD19 Luciferase CHO cells (BPS Bioscience, #79714) is ~10 fold higher than from Luciferase CHO cells (BPS Bioscience, #79725). This is due to the different expression level of luciferase in these two cell lines, and does not affect the performance of the coculture assay.

IFNγ analysis: The IFNγ expression of each well can be determined using the Colorimetric Human IFN-γ ELISA Detection Kit (BPS Bioscience, #79777), following the recommended protocol. If the IFNγ assay is not performed immediately, the collected medium can be stored at -20°C.

License Disclosure

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

Troubleshooting Guide

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

Figures and Validation Data

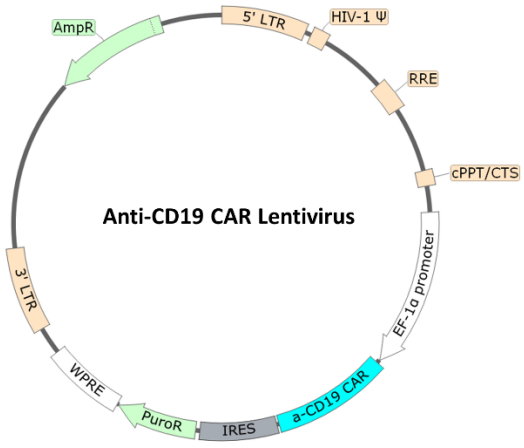

Figure 1A. Schematic of the lenti-vector used to generate the anti-CD19 CAR lentivirus

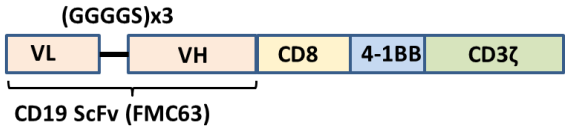

Figure 1B. Construct showing components of anti-CD19 CAR

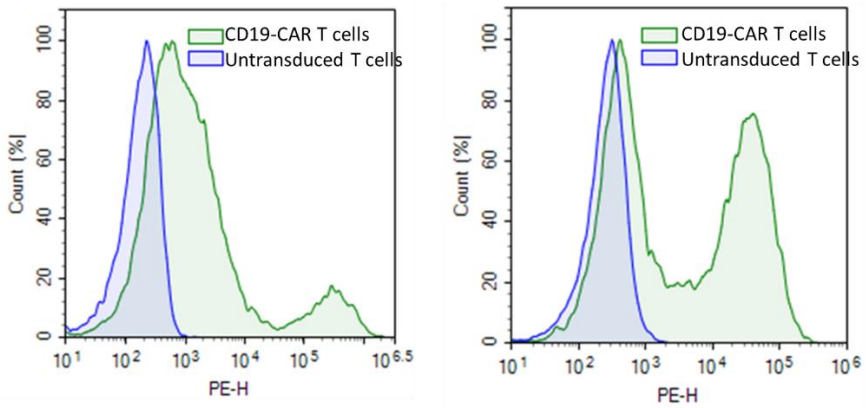

Figure 2. The expression of anti-CD19 CAR in T cells transduced with anti-CD19 CAR lentivirus
Approximately 15,000 CD4+CD8+ activated T cells were transduced with 600,000 TU (at MOI of 40) anti-CD19 CAR Lentivirus in the presence of 5 µg/mL of polybrene via spinoculation. The anti-CD19 CAR expression was analyzed by flow cytometry using PE-anti-FMC63 ScFv (Acrobiosystems, #FM3-HPY53-25tests). A) transduced T cells analyzed 72 hours post-transduction (transient expression); B) transduced T cells analyzed 10 days post transduction (stable integration). Green, anti-CD19 CAR lentivirus transduced T cells; Blue, Untransduced T cells.

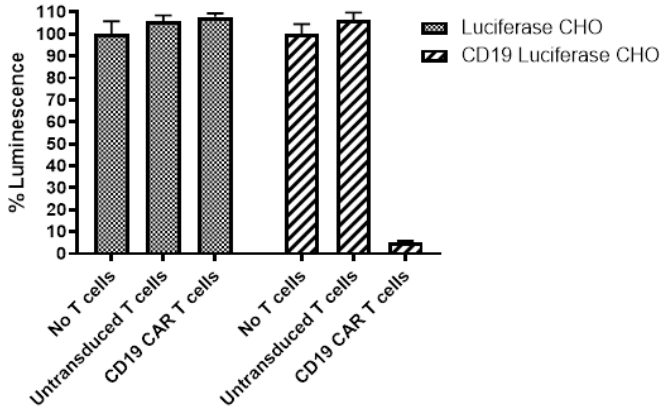

Figure 3. Luciferase-based cytotoxicity assay

Approximately 15,000 CD4+CD8+ activated T cells were transduced with 600,000 TU (at MOI of 40) anti-CD19 CAR Lentivirus in the presence of 5 µg/mL of polybrene via spinoculation. Transduced T cells were expanded. Twelve days post-transduction, the T cells (effector) were co-cultured with Luciferase CHO cell or CD19/Luciferase CHO cells (target) for 24 hours at a ratio of effector: target=20. The lysis of target cells was determined by measuring Luciferase activity. The anti-CD19 CAR lentivirus transduced T cells showed specific toxicity towards CD19/Luciferase CHO cells. The assay was performed in parallel with untransduced T cells as a negative control.

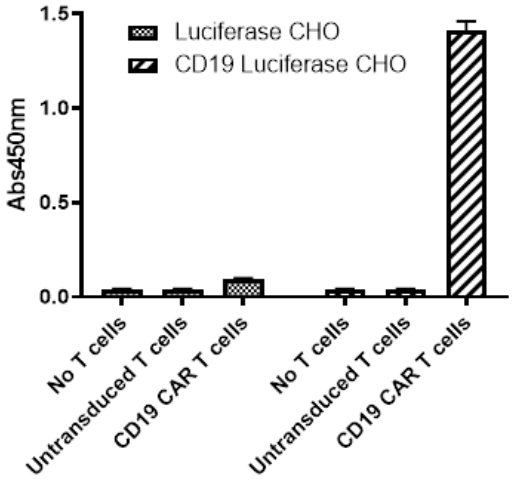

Figure 4. IFNγ expression analysis

Approximately 15,000 CD4+CD8+ activated T cells were transduced with 600,000 TU (at MOI of 40) anti-CD19 CAR Lentivirus in the presence of 5 µg/mL of polybrene via spinoculation. Transduced T cells were expanded. Twelve days post-transduction, the T cells (effector) were co-cultured with Luciferase CHO cell or CD19/Luciferase CHO cells (target) for 24 hours at a ratio of effector: target=20. The medium was then collected for IFNγ analysis using IFN-γ ELISA Detection Kit (BPS Bioscience, #79777).

Related Products

Products	*Catalog #*	*Size*
CD19 / Firefly Luciferase - CHO Recombinant Cell Line	79714	2 vials
Firefly Luciferase - CHO Recombinant Cell Line	79725	2 vials
ONE-Step™ Luciferase Assay System	60690	Multiple
IFN-γ (Human) Colorimetric ELISA Detection Kit	79777	96 reactions
Human Interleukin-2	90184-A	10 µg
Normal Human Peripheral Blood Mononuclear Cells	79059	30 x 10^6 cells
Anti-CD19 CAR / NFAT (Luciferase) Reporter Jurkat Cell Line (CD19 SCFV-CD28-4-1BB-CD3ζ)	79853	2 vials
Anti-CD19 CAR negative control/ NFAT (Luciferase) Reporter Jurkat Cell Line	79854	2 vials
Anti-BCMA CAR /NFAT (Luciferase) Reporter Jurkat Cell Line	79694	2 vials
CD19 / CD20 / Firefly Luciferase CHO Cell Line	78186	2 vials
CD19, Fc-Fusion (IgG1), Avi-Tag, PE-labeled	100732	50 µg
Anti-CD19 IgG Antibody	100981	50 µg
CD19 CHO Recombinant Cell Line (Low, Medium and High Expression)	79561	2 vials
Anti-CD19-Anti-CD3 Bispecific Antibody	100441	50 µg
Firefly Luciferase Lentivirus (Hygromycin)	79692-H	500 µl x 2
Firefly Luciferase Lentivirus (Puromycin)	79692-P	500 µl x 2
NFAT Luciferase Reporter Lentivirus	79579	500 µl x 2