Description

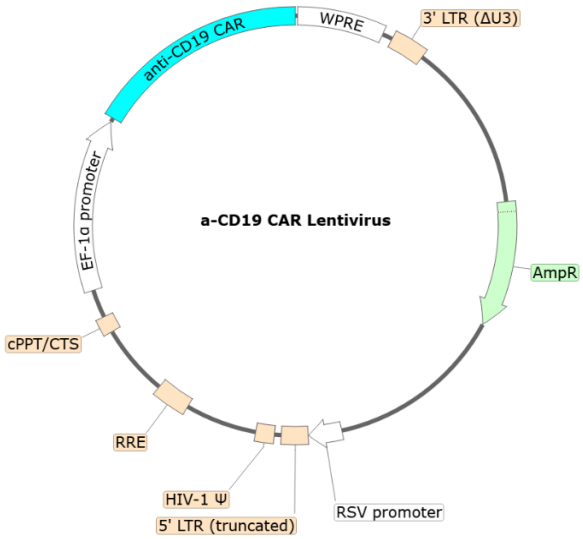
The anti-CD19 CAR lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to infect almost all types of mammalian cells, including primary and non-dividing cells. These viruses transduce the ScFv portion of anti-CD19 (clone FMC63) linked to 2nd generation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

Note: This product transduces the same construct as the anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ) (BPS Bioscience #78600), but differs in key aspects:

1) 78601, described here, is constructed with a SIN lentivector while 78600 is not.
2) 78601, described here, does not contain an antibiotic for selection while 78600 contains a puromycin selection so the transduced cells can be selected with puromycin.

BPS Bioscience CAT #	Self-Inactivation (SIN)	Antibiotic selection
78601	yes	no
78600	no	puromycin

A.



B.

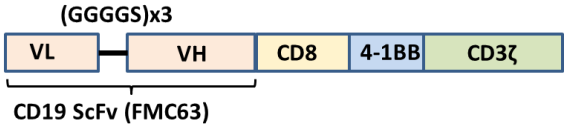


Figure 1. (A) Schematic of the lenti-vector used to generate the anti-CD19 CAR lentivirus. The vector is a SIN vector, and it does not contain a mammalian selection antibiotic. (B) Construct diagram showing components of the anti-CD19 CAR.

Application

- Ideal as a positive control for anti-CD19 CAR evaluation in T cells
- Use in transduction optimization
- Use to generate anti-CD19 CAR-T cells for research use only, not for therapeutic purposes

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

The lentiviruses 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. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience 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 Antibody	Acrobiosystems, # FM3-HPY53-25tests
CD19 / Firefly Luciferase - CHO Recombinant Cell Line	BPS Bioscience #79714
Firefly Luciferase - CHO Recombinant Cell Line	BPS Bioscience #79725
Firefly Luciferase Raji Cell Line	BPS Bioscience #78622
Firefly Luciferase K562 Cell Line	BPS Bioscience #78621
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690

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 protocol was used to transduce CD4+CD8+ primary T cells with the 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: CD4+ T cells and CD8+ T cells were isolated from previously frozen human PBMC by negative selection, according to the manufacturer's instructions. The isolated CD4+ T cells and CD8+ T cells were mixed at a 1:1 ratio and cultured using the recommended T cell medium at 1 x 10^6 cells/ml density. The cells were incubated at 37°C with 5% CO_2 overnight.

2. Day 1: T cell activation reagents were added to the cells and incubated at 37°C with 5% CO_2 for 24-48 hours.

3. Day 2: The T cells were centrifuged (300 x g for 5 minutes) and resuspended in fresh T cell medium at 0.1-0.2 x 10^6 cells/ml. Polybrene (5 µg/ml) was added to the cells.

   The anti-CD19 CAR lentivirus was thawed on ice. Note: Lentiviruses are very sensitive to freeze/thaw cycles. Following the first thaw, prepare small aliquots of virus to limit cycles of freeze/thaw.

   Spinoculation:

   1) 100 µl of T cells (~10,000-20,000) were distributed into 1.5 ml Eppendorf tubes.

   2) The MOI was titrated starting from 20. The lentivirus was incubated with the cells in the hood at room temperature for 10 minutes. The cells/virus mixes were spun gently at 800 x g for 2 hours at 32°C.

   3) 900 µl of fresh T cell medium was added into each well of a 24-well plate. The cells/virus mixes from the spinoculation step were added to the 24-well plate. It was not necessary to remove the virus. The cells were incubated at 37°C with 5% CO_2 for ~48-72 hours.

4. Day 5: The expression of the anti-CD19 CAR was estimated by flow cytometry using PE-Labeled anti-FMC63 scFv antibody. The remaining transduced T cells were expanded further using the recommended T cell medium.

   Note: 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 the experience of scientists at BPS Bioscience, the T cells had expanded >1000 fold by 11 days post-transduction, using the recommended T cell medium.

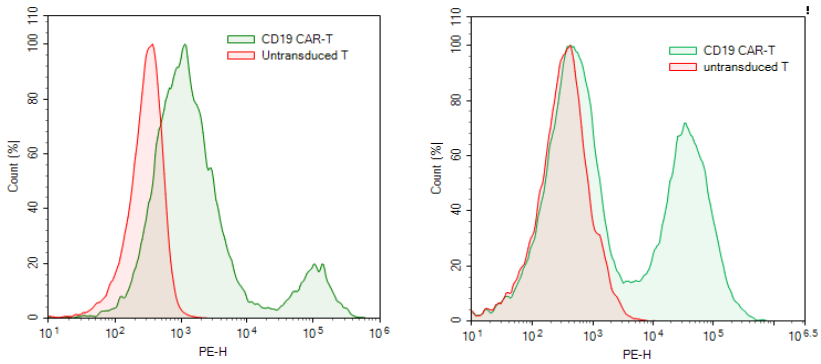


Figure 2. Expression of anti-CD19 CAR in T cells transduced with anti-CD19 CAR lentivirus. Approximately 15,000 CD4+CD8+ activated T cells were transduced with 600,000 TU (at MOI of 40) anti-CD19 CAR Lentivirus in the presence of 5 µg/ml of polybrene via spinoculation. Anti-CD19 CAR expression was analyzed by flow cytometry using PE-anti-FMC63 ScFv (Acrobiosystems, #FM3-HPY53-25tests) 72 hours post-transduction (left) or 12 days post-transduction (right). Green: anti-CD19 CAR lentivirus transduced T cells; Red: Untransduced T cells.

The following experiments are two examples of co-culture assays performed to evaluate the cytotoxicity of anti-CD19 CAR-T using CD19/Firefly Luciferase CHO Cell Line or Firefly Luciferase Raji Cell Line as the target cells.

Cytotoxicity assay using CD19/Firefly Luciferase CHO Cell Line as the target cells

1. Day 12: Target cells "CD19/Firefly Luciferase CHO Cell Line" (BPS Bioscience #79714) and negative control "Firefly Luciferase CHO Cell Line" (BPS Bioscience #79725) were seeded in 50 µl of Thaw Medium 3 (BPS Bioscience #60186) at 500 cells/well in a 96-well white, clear bottom tissue culture plate.

    1) Extra wells of CD19/Firefly Luciferase CHO cells or Firefly Luciferase CHO cells were included for the "no T cell" control.

    2) Extra wells of medium only were included to determine background luminescence.

    The transduced T cells were centrifuged gently (300 x g for 5 minutes), resuspended in fresh T cell growth medium, and were carefully pipetted into each well at the desired effector:target (E:T) cell ratio in a volume of 50 µl. For "no T cells" wells and "medium only" wells, 50 µl of fresh T cell medium was added. The total volume of each well was 100 µl. The plates were incubated at 37°C for 24 hours.

    Note: No overnight attachment was needed for the CHO cells. T cells were added into the wells 1-2 hours after the CHO cells were seeded.

2. Day 13: Each well was pipetted gently up and down 3 to 4 times. The medium containing the non-attached cells was transferred to another plate.

    Luciferase assay was performed using the CHO cells remaining on the plate whereas the collected medium can be subjected to cytokine release analysis**.** If the cytokine release analysis is not performed immediately, the collected medium can be stored at -20°C.

Luciferase assay: The ONE-Step™ Luciferase reagent (BPS Bioscience, #60690) was prepared following the recommended protocol and 50 µl of ONE-Step™ Luciferase assay reagent was added to each well, including empty wells (that had contained medium only) to determine the background luminescence. The plate was incubated at room temperature 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 Luciferase CHO cells or CD19/Luciferase CHO 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 cells" control wells (Luciferase CHO or CD19 Luciferase CHO cells only).

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

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.

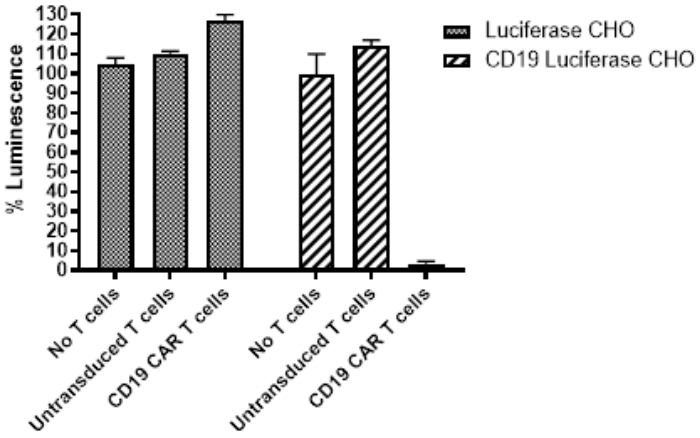


Figure 3. Luciferase-based cytotoxicity assay using CD19/Firefly Luciferase CHO cells as the target cells. Approximately 15,000 CD4+CD8+ T cells were transduced with 600,000 TU (at MOI of 40) anti-CD19 CAR Lentivirus in the presence of 5 µg/ml of polybrene via spinoculation. The transduced T cells were expanded. Twelve days post-transduction, the T cells (effector) were co-cultured with Firefly Luciferase CHO cell or CD19/Firefly Luciferase CHO cells (target) for 24 hours at an effector:target ratio of 20. The lysis of the target cells was determined by measuring Luciferase activity. The anti-CD19 CAR lentivirus-transduced T cells showed specific toxicity towards CD19/Firefly Luciferase CHO cells. The assay was performed in parallel with untransduced T cells as a negative control.

Cytotoxicity assay using Firefly Luciferase Raji Cell Line as the target cells

1. Day 12: Target cells "Firefly Luciferase Raji Cell Line" (BPS Bioscience #78622) and negative control "Firefly Luciferase K562 Cell Line (BPS Bioscience #78621), which do not express CD19, were seeded in 50 µl of Thaw Medium 2 (BPS Bioscience #60184) at 5000 cells/well in a 96-well white, clear bottom tissue culture plate.

1) Extra wells of Firefly Luciferase Raji cells or Firefly Luciferase K562 cells were included for the "no T cell" control wells.

2) Extra wells of medium only were included to determine background luminescence.

The transduced T cells were centrifuged gently (300 x g for 5minutes), resuspended in fresh T cell growth medium, and were carefully pipetted into each well at the desired effector:target (E:T) cell ratio in a volume of 50 µl. For "no T cells" wells and "medium only" wells, 50 µl of fresh T cell medium was added. The total volume of each well was 100 µl. The plates were incubated at 37°C for 24 hours.

2. Luciferase assay: The ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was prepared following the recommended protocol and 100 µl of ONE-Step™ Luciferase assay reagent was added to each well, including empty wells (that had contained medium only) to determine the background luminescence. The plate was incubated at room temperature 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 Raji Recombinant Cell Line or Firefly Luciferase K562 Cell Line was set as 100%. The % Luminescence was calculated as background-subtracted luminescence of co-culture wells divided by background-subtracted luminescence from the "no T cells" control wells (Luciferase Raji or Luciferase K562 cells only). Firefly Luciferase K562 cells (BPS Bioscience #78621), which do not express endogenous CD19, were used as a negative control.

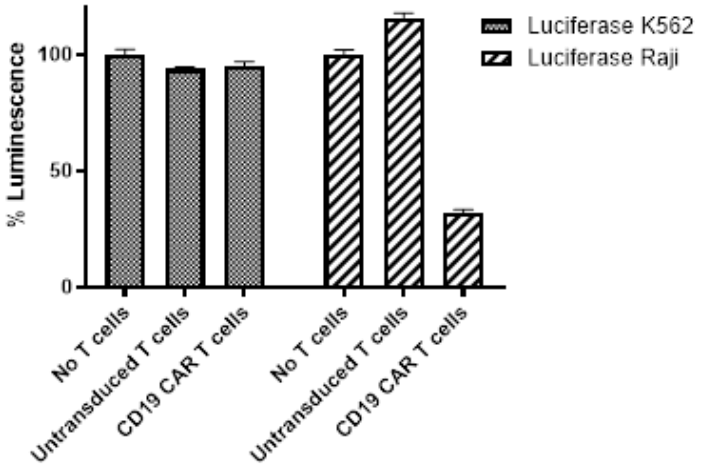


Figure 4. Luciferase-based cytotoxicity assay using Firefly Luciferase Raji as the target cells. Approximately 15,000 CD4+CD8+ T cells were transduced with 600,000 TU (at MOI of 40) anti-CD19 CAR Lentivirus in the presence of 5 µg/mL of polybrene via spinoculation. Transduced T cells were expanded. Twelve days post-transduction, the T cells (effector) were co-cultured with Firefly Luciferase Raji cell or Firefly Luciferase K562 cells (target) for 24 hours at a ratio of effector:target of 5. The lysis of target cells was determined by measuring Luciferase activity. The assay was performed in parallel with untransduced T cells and Firefly Luciferase K562 Cells as negative controls.

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 troubleshooting instructions or email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Untransduced T cells (Negative Control for CAR-T Cells)	78170	1 vial
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
Anti-BCMA CAR Lentivirus (Clone C11D5.3 ScFv-CD8-CD28-CD3ζ)	78603	50 µl
Anti-CD20 CAR Lentivirus (Clone Leu-16 ScFv-CD8-4-1BB-CD3ζ)	78606	50 µl
Anti-CD22 CAR Lentivirus (Clone m971 ScFv-CD8-4-1BB-CD3ζ)	78608	50 µl
Anti-CD19/CD22 Bispecific CAR Lentivirus (Clones FMC63/m971 ScFv-CD8-4-1BB-CD3ζ)	78609	50 µl