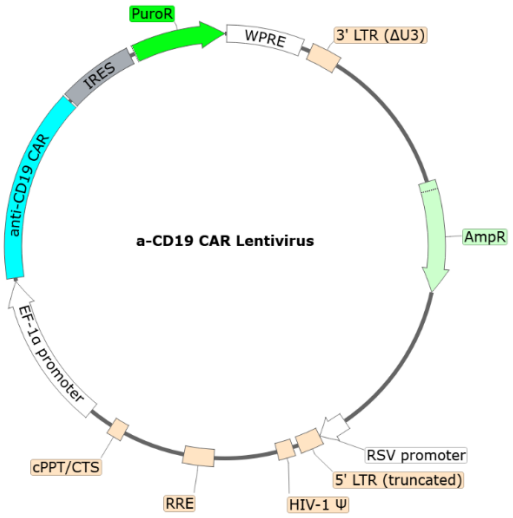
Description

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

A.



B.

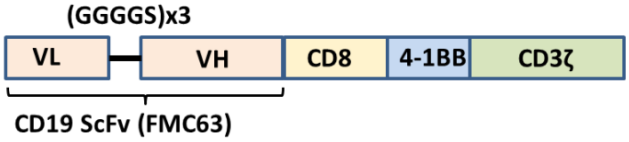


Figure 1. (A) Schematic of the lenti-vector used to generate the anti-CD19 CAR lentivirus. The vector is a SIN vector, and it contains a puromycin selection marker. (B) Construct diagram showing components of the anti-CD19 CAR.

Note: This product transduces the same anti-CD19 CAR construct (CD19 ScFv-CD8-4-1BB-CD3ζ) as other available BPS Bioscience anti-CD19 CAR lentiviruses (BPS Bioscience #78600,78601 and 78775) but differs in key aspects. This product contains a puromycin selection marker that can be used for selection. Please see the table below.

BPS Bioscience Catalog #	Self-Inactivation (SIN)	Selection Marker
78600	no	puromycin
78601	yes	no
78602	yes	puromycin
78775	yes	eGFP

Background

CD19 (also known as Cluster of Differentiation 19, B-lymphocyte surface antigen B4, or CVID3) is a glycoprotein expressed at the surface of B lymphocytes through most phases of B cell maturation. It is strictly required for B cell terminal differentiation. Mutations in the CD19 gene cause severe immune-deficiency syndromes associated with impaired antibody production such as CVID3 (common variable immuno-deficiency 3). The majority of B cell malignancies express normal to high levels of CD19, making it a nearly ideal target for cancer immunotherapy. Blinatumomab, a CD19/CD3 bi-specific T cell engager (BiTE) has been approved for relapsed/refractory B precursor ALL (Acute lymphoblastic leukemia) and CD19 was the target of the first approved CAR-T cell therapy. Studies of CD19 function and expression profiles will continue to broaden our knowledge and support broader applications in cancer therapy.

Application

- Positive control for anti-CD19 CAR evaluation in T cells.
- Transduction optimization experiments.
- 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. Virus particles can be packaged in custom formulations by special request, for an additional fee.

Size and Titer

50 µl of anti-CD19 CAR at a titer ≥ $3x10^8$ 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 a 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. 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 protocol described in the next section. 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

Media Formulations

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

Media Required for the Proposed Assay

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.

Thaw Medium 2 (BPS Bioscience #60184):

RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

Assay Protocol

The following protocol was used to transduce CD4$^+$ and CD8$^+$ primary T cells with the anti-CD19 CAR Lentivirus, and it is a general guideline only. The transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type, donor and the assay requirements.

Day 0:

1. Isolate CD4$^+$ T cells and CD8$^+$ T cells from previously frozen human PBMC by negative selection, according to the manufacturer's instructions. Mix the isolated CD4$^+$ T cells and CD8$^+$ T cells at a 1:1 ratio.

2. Culture cells in TCellM™ at 1 x 10^6 cells/ml density, at 37°C with 5% CO_2 overnight.

Day 1:

1. Activate T cells with the appropriate reagents and incubate at 37°C with 5% CO_2 for 24 - 48 hours.

Day 2:

1. Centrifuge the cells at 300 x *g* for 5 minutes and resuspend in fresh T cell medium at 0.1 - 1 x 10^6 cells/ml.

2. Add polybrene (5 µg/ml) to the cells.

3. Thaw anti-CD19 CAR lentiviruses 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.

4. Perform spinoculation, as follows:
   a) Dispense 100 µl of T cells (~10,000-100,000 cells) into each 1.5 ml Eppendorf tube.

   b) Create a titration of the viruses MOI starting from a MOI of 10.

   c) Incubate in a hood at Room Temperature (RT) for 10 minutes.

   d) Spin down the cells/virus at 800 *x g* for 2 hours at 32°C.

   e) If using 10,000 cells: add 900 µl of fresh T cell medium into each well of a 24-well plate, followed by the cells/virus from the spinoculation step.

      If using 100,000 cells: add 3 ml of fresh T cell medium into each well of a 6-well plate, followed by the cells/virus from the spinoculation step.

5. It is not necessary to remove the virus after spinoculation. Incubate the cells at 37°C with 5% CO_2 for ~48-72 hours.

Day 5:

1. Analyze the expression of the anti-CD19 CAR by flow cytometry, using PE-Labeled anti-FMC63 scFv antibody, as shown in Figure 2. The remaining transduced T cells can be expanded further using TCellM™. Please see below for details on further assays performed at later days.

   Note: Once the transduced cells have proliferated sufficiently to reach the desired cell number required for your experiments it is recommended the cells are used, in order to minimize cellular exhaustion. In the experience of scientists at BPS Bioscience, when using TCellM™ T cells can expand >1000 fold by day 11 post-transduction.

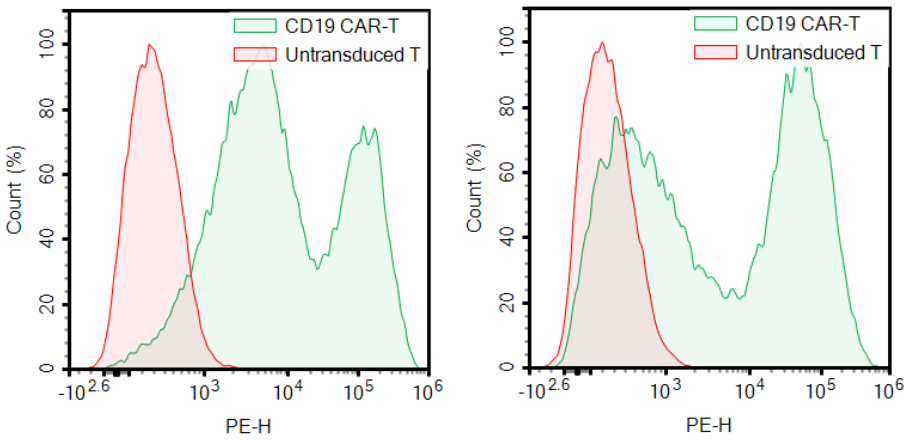


Figure 2. Expression of anti-CD19 CAR in T cells transduced with anti-CD19 CAR lentivirus. Approximately 15,000 $CD4^+$ and $CD8^+$ activated T cells were transduced with 300,000 TU (MOI of 20) anti-CD19 CAR lentivirus in the presence of 5 µg/ml of polybrene, by spinoculation. Anti-CD19 CAR expression 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).

The following experiments are two examples of co-culture assays that can be performed to evaluate the cytotoxicity of anti-CD19 CAR-T using the CD19/Firefly Luciferase CHO Cell Line (BPS Bioscience #79714) or Firefly Luciferase Raji Cell Line (BPS Bioscience #78622) as the target cells. Firefly Luciferase CHO Cell Line (BPS Bioscience #79725) and Firefly Luciferase K562 Cell Line (BPS Bioscience #78621) should be used as negative control.

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

The assay should include a "No T cell Control", "Background Luminescence Control" and "Test" conditions.

Day 12:

1. Seed the target cells (CD19/Firefly Luciferase CHO Cells) and negative control cells (Firefly Luciferase CHO Cell Line) in 50 µl of Thaw Medium 3 at 500 cells/well in a 96-well white, clear bottom tissue culture plate. These are the "Test" wells. Include extra wells of CD19/Firefly Luciferase CHO cells or Firefly Luciferase CHO cells as "No T cell Control", and wells containing only media as "Background Luminescence Control".

2. After 1-2 hours proceed with the protocol.

   Note: No overnight attachment is needed for the CHO cells.

3. Centrifuge the transduced T cells gently at 300 *x g* for 5 minutes.

4. Resuspend T cells in fresh TCellM™.

5. Carefully pipet 50 µl of T cells, at the appropriate density to reach the desired effector:target (E:T), into each "Test" well.

6. For "No T cell Control" and "Background Luminescence Control" wells add 50 µl of fresh TCellM™.

7. Incubate at 37°C for 24 hours.

Day 13:

1. Pipet each well gently up and down 3 to 4 times.

2. Transfer the medium containing the non-attached cells to another plate.

   Note: 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.

3. Add 50 µl of ONE-Step™ Luciferase assay reagent to each well, including the control wells.

4. Incubate the plate at RT for ~15 to 30 minutes.

5. Measure luminescence using a luminometer.

Data Analysis: Subtract the average background luminescence from the luminescence reading of all wells. Set the luciferase activity of CD19/Luciferase CHO cells or Luciferase CHO cells alone as 100%. The % Luminescence (Lum) was calculated by dividing the background-subtracted luminescence of co-culture wells by the 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 levels of luciferase in the two cell lines and does not affect the performance of the co-culture assay.

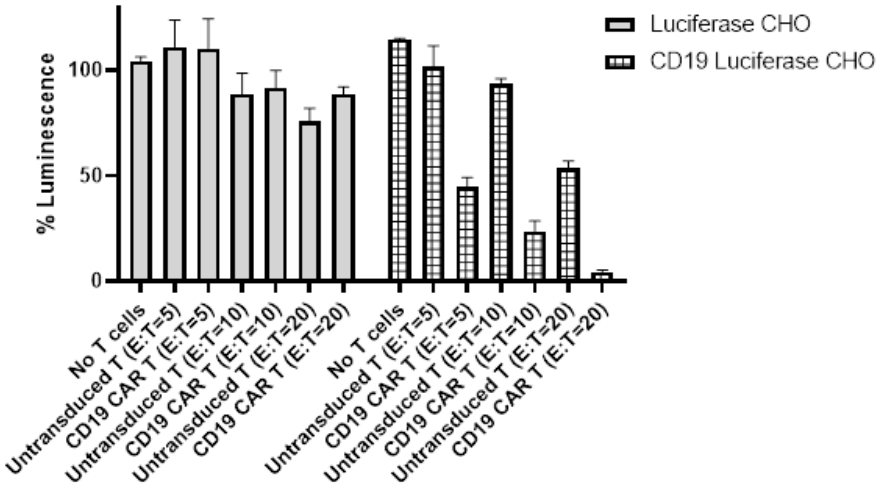


Figure 3. Cytotoxicity profile of T cells transduced with anti-CD19 CAR Lentivirus against CD19/Firefly Luciferase CHO cells target cells.
Approximately 15,000 $CD4^+$ and $CD8^+$ T cells were transduced with 300,000 TU (MOI of 20) anti-CD19 CAR Lentivirus in the presence of 5 µg/ml of polybrene, by spinoculation, and expanded. Twelve days post-transduction, the T cells (effector) were co-cultured with Firefly Luciferase CHO cells or CD19/Firefly Luciferase CHO cells (target) for 24 hours at different E:T ratios. The death of the target cells was determined by measuring luciferase activity with ONE-Step™ Luciferase. The anti-CD19 CAR lentivirus-transduced T cells showed specific cytoxicity towards CD19/Firefly Luciferase CHO cells even at an E:T ratio of 5 (lower % luminescence). Untransduced T cells were run in parallel as a negative control. The luciferase activity of CD19/Luciferase CHO cells or Luciferase CHO cells alone was set as 100%.

Cytotoxicity assay using Firefly Luciferase Raji Cell Line as the target cells.
The assay should include a "No T cell Control", "Background Luminescence Control" and "Test" conditions.

Day 12:

1. Seed the target cells (Firefly Luciferase Raji Cell Line) and negative control cells (Firefly Luciferase K562 Cell Line, which do not express CD19), in 50 µl of Thaw Medium 2 at 5000 cells/well in a 96-well white, clear bottom tissue culture plate. Include extra wells of Firefly Luciferase Raji cells or Firefly Luciferase K562 cells as "No T cell Control", and wells containing medium only as "Background Luminescence Control".

2. Centrifuge transduced T cells gently at 300 *x g* for 5 minutes.

3. Resuspend transduced T cells in fresh TCellM™.

4. Carefully pipet 50 µl of T cells, at the appropriate density to reach the desired effector:target (E:T), into each "Test" well.

5. For "No T cell Control" and "Background Luminescence Control" wells add 50 µl of fresh TCellM™.

6. Incubate at 37°C for 24 hours.

Day 13:

1. Add 100 µl of ONE-Step™ Luciferase assay reagent to each well, including the control wells.

2. Incubate the plate at RT for ~15 to 30 minutes.

3. Measure 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 Cell Line or Firefly Luciferase K562 Cell Line was set as 100%. The % Luminescence was calculated by dividing background-subtracted luminescence of co-culture wells by the background-subtracted luminescence from the "no T cells Control" wells (Luciferase Raji or Luciferase K562 cells only). Firefly Luciferase K562 cells, which do not express endogenous CD19, were used as a negative control.

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

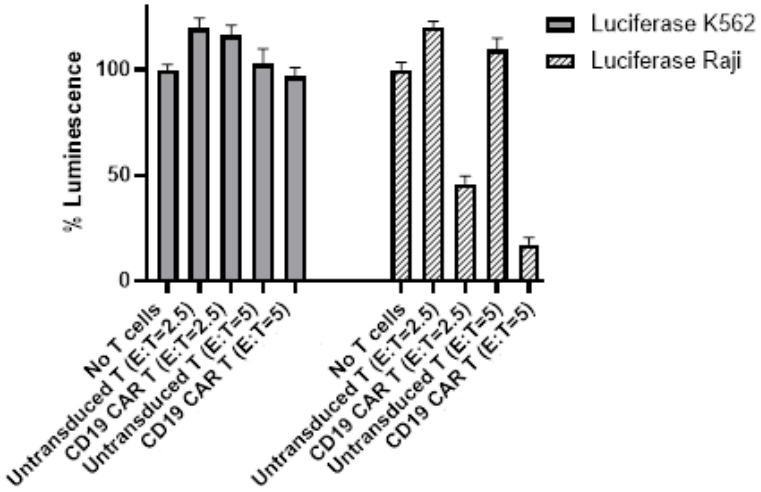


Figure 4. Cytotoxicity profile of T cells transduced with anti-CD19 CAR Lentivirus against Firefly Luciferase Raji target cells.
Approximately 15,000 $CD4^+$ and $CD8^+$ T cells were transduced with 300,000 TU (MOI of 20) anti-CD19 CAR Lentivirus in the presence of 5 µg/ml of polybrene, by spinoculation, and 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 different E:T ratios. The death of target cells was determined by measuring luciferase activity with ONE-Step™ Luciferase. The anti-CD19 CAR lentivirus-transduced T cells showed specific cytoxicity towards CD19/Firefly Luciferase Raji cells even at an E:T ratio of 2.5 (lower % luminescence). Untransduced T cells were run in parallel as a negative control. The luciferase activity of Luciferase K562 cells or Luciferase Raji cells alone was set as 100%.

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

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.

Related Products

Products	*Catalog #*	*Size*
Untransduced T cells (Negative Control for CAR-T Cells)	78170	1 vial
$CD4^+$ T cells, Negatively Selected (Human)	79752	10 million cells
$CD8^+$ T Cells, Negatively Selected (Human)	79753	10 million cells
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-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ, eGFP)	78775	50 µl
Anti-CD19/CD22 Bispecific CAR Lentivirus (Clones FMC63/m971 ScFv-CD8-4-1BB-CD3ζ)	78609	50 µl