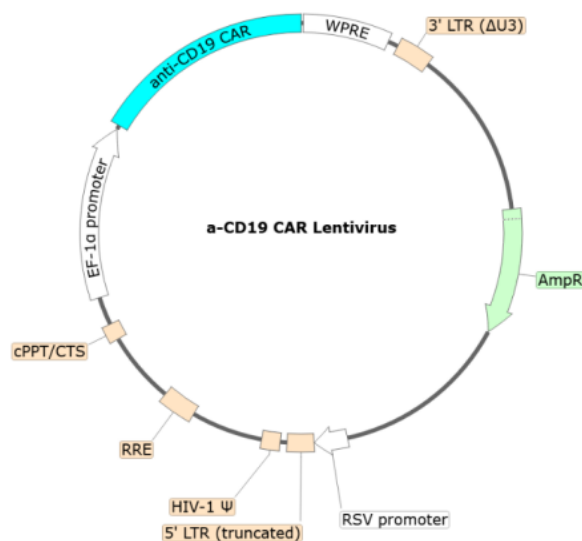


## Description

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 the ScFv portion of anti-CD19 (clone FMC63) linked to 2<sup>nd</sup> generation CAR (Chimeric Antigen Receptor), containing CD8 hinge, 4-1BB and CD3 $\zeta$  signaling domains (Figure 1).

A.



B.

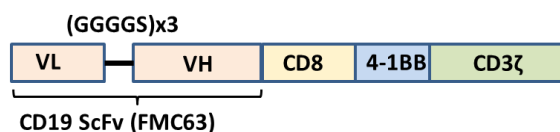


Figure 1. (A) Schematic of the lenti-vector used to generate the anti-CD19 CAR lentivirus. This vector is a SIN vector. No mammalian selection marker is present. (B) Construct diagram showing components of the anti-CD19 CAR.

**Note:** This product transduces the same anti-CD19 CAR construct (CD19 ScFv-CD8-4-1BB-CD3 $\zeta$ ) as other available anti-CD19 CAR Lentiviruses (BPS Bioscience # 78600, 78602 and 78775), but differ in key aspects. Please see the table below.

BPS Bioscience #	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

One vial (50  $\mu$ l) of anti-CD19 CAR at a titer  $\geq 3 \times 10^8$  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. 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	<a href="#">BPS Bioscience #79059</a>
Human Interleukin-2	<a href="#">BPS Bioscience #90184</a>
EasySep™ Human CD4 <sup>+</sup> T Cell Isolation Kit	Stemcell technologies, #17952
EasySep™ Human CD8 <sup>+</sup> 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
CD19 / Firefly Luciferase - CHO Recombinant Cell Line	<a href="#">BPS Bioscience #79714</a>
Firefly Luciferase - CHO Recombinant Cell Line	<a href="#">BPS Bioscience #79725</a>
Firefly Luciferase Raji Cell Line	BPS Bioscience #78622
Firefly Luciferase K562 Cell Line	BPS Bioscience #78621
ONE-Step™ Luciferase Assay System	<a href="#">BPS Bioscience #60690</a>

**Media Formulations**

T Cell Medium: TCellIM™ (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.

**Assay Protocol**

The following protocol was used to transduce CD4<sup>+</sup> and CD8<sup>+</sup> primary T cells using the anti-CD19 CAR Lentivirus and 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<sup>+</sup> T cells and CD8<sup>+</sup> T cells from previously frozen human PBMC by negative selection, according to the manufacturer's instructions. Mix the CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells at a 1:1 ratio.
2. Culture cells in TCellIM™ at 1 x 10<sup>6</sup> cells/ml density, at 37°C with 5% CO<sub>2</sub> overnight.

**Day 1:**

1. Activate T cell with the appropriate reagents and incubate at 37°C with 5% CO<sub>2</sub> for 24 - 48 hours.

**Day 2:**

1. Centrifuge the cells at 300 x g for 5 minutes and resuspended in fresh T cell medium at 0.1-0.2 x 10<sup>6</sup> cells/ml.
2. Add polybrene (5 µg/ml) to the cells.

3. Thaw anti-CD19 CAR lentiviruses on ice.

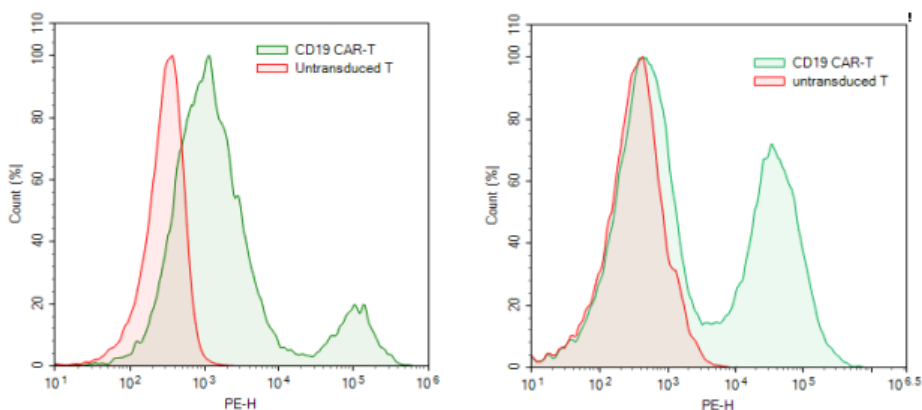
*Note: Lentiviruses are very sensitive to freeze/thaw cycles. Following the first thaw, prepare small aliquots of virus to limit cycles of freeze/thaw.*

4. Perform spinoculation, as follows:
  - a) Dispense 100  $\mu$ l of T cells (~10,000-20,000 cells) into 1.5 ml Eppendorf tubes.
  - b) Create a titration of the viruses MOI starting from a MOI of 20.
  - 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) Add 900  $\mu$ l of fresh T cell medium into each well of a 24-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<sub>2</sub> 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 TCellIM™. 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 TCellIM™ 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<sup>+</sup> and CD8<sup>+</sup> activated T cells were transduced with 600,000 TU (MOI of 40) anti-CD19 CAR Lentivirus in the presence of 5  $\mu$ g/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) and 12 days post-transduction (right).

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

The following experiments are two example of co-assays that can be performed to evaluate the cytotoxicity of anti-CD19 CAR-T cells using the CD19/Firefly Luciferase CHO Cell Line (BPS Bioscience #79714) and Firefly 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 controls.

The assay should include “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  $\mu$ l 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 TCellIM™.
5. Carefully pipet 50  $\mu$ l 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  $\mu$ l of fresh TCellIM™.
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  $\mu$ l 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.

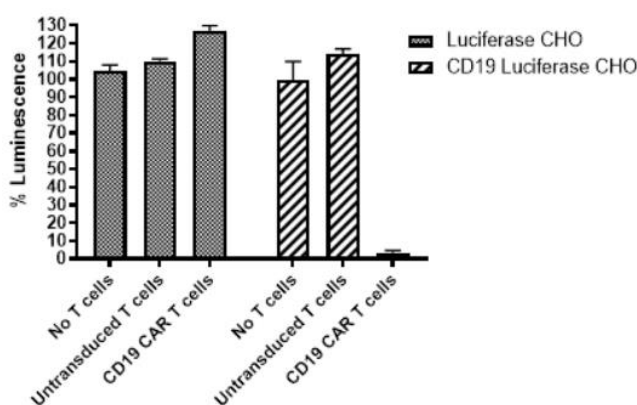


Figure 3. Cytotoxicity profile of T cells transduced with anti-CD19 CAR Lentivirus against CD19/Firefly Luciferase CHO cells target cells.

Approximately 15,000 CD4<sup>+</sup> and CD8<sup>+</sup> activated T cells were transduced with 600,000 TU (MOI of 40) anti-CD19 CAR Lentivirus in the presence of 5  $\mu$ g/ml of polybrene, by spinoculation, and expanded. Twelve days post-transduction, the T cells (effector) were co-cultured with Luciferase CHO cells or CD19/Luciferase CHO cells (target) for 24 hours at a ratio of effector:target of 20. The lysis of the target cells was determined by measuring luciferase activity with ONE-Step™ Luciferase. The anti-CD19 CAR lentivirus transduced T cells showed specific cytotoxicity towards CD19/Luciferase CHO cells. 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  $\mu$ l 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.

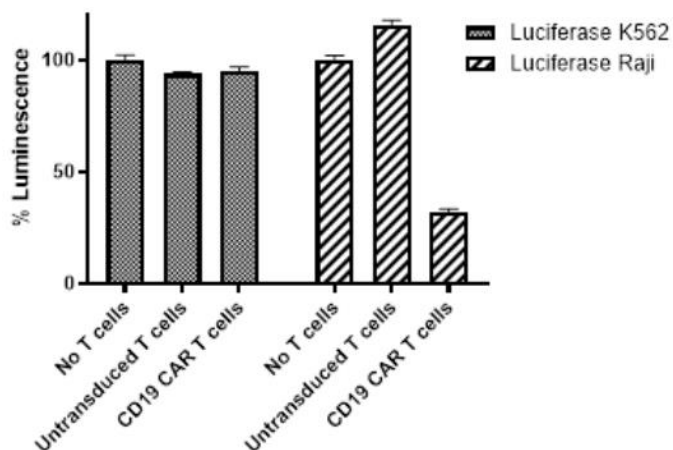
3. Resuspend transduced T cells in fresh TCellIM™.
4. Carefully pipet 50 µl of T cells, at the appropriate density to reach the desired effector:target (E:T), into each “Test” well.
5. For “No T cell Control” and “Background Luminescence Control” wells add 50 µl of fresh TCellIM™.
6. Incubate at 37°C 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 alone 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 CD19/Firefly Luciferase Raji cells target cells.*

Approximately 15,000 CD4<sup>+</sup> and CD8<sup>+</sup> activated T cells were transduced with 600,000 TU (MOI of 40) anti-CD19 CAR Lentivirus in the presence of 5  $\mu$ g/mL of polybrene, by spinoculation, and expanded. Twelve days post-transduction, the T cells (effector) were co-cultured with Firefly Luciferase Raji or Firefly Luciferase K562 cells (target) for 24 hours at a ratio of effector:target of 5. The lysis of the target cells was determined by measuring luciferase activity with ONE-Step™ Luciferase. The anti-CD19 CAR lentivirus transduced T cells showed specific cytotoxicity towards Luciferase Raji cells. Untransduced T cells were run in parallel as a negative control. The luciferase activity of Luciferase Raji cells or Luciferase K562 cells alone was set as 100%.

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

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#### Troubleshooting Guide

Visit [bpsbioscience.com/lentivirus-faq](https://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 <sup>+</sup> T cells, Negatively Selected (Human)	79752	10 million cells
CD8 <sup>+</sup> T Cells, Negatively Selected (Human)	79753	10 million cells
Anti-CD19 CAR-T cells	78171	1 vial