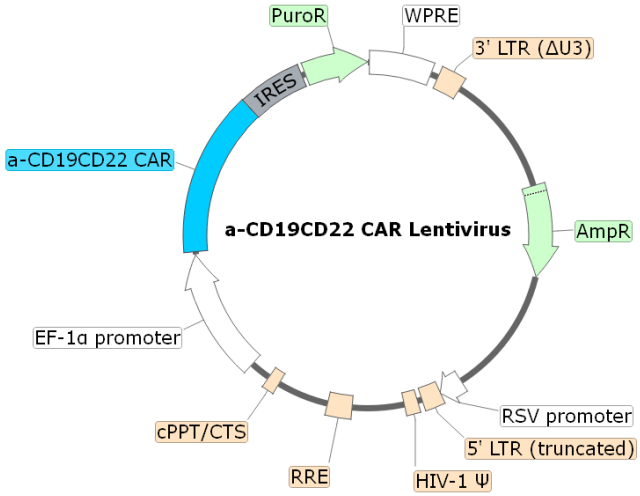
Description

The anti-CD19/CD22 Bispecific 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 (single-chain variable fragments) of anti-CD19 (clone FMC63) and anti-CD22 (clone m971) linked to a 2nd generation CAR (Chimeric Antigen Receptor) containing CD8 hinge and transmembrane domains, and the 4-1BB and CD3ζ signaling domains (Figure 1).

A.



B.

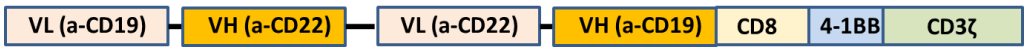


Figure 1. *(A) Schematic of the lenti-vector used to generate the anti-CD19/CD22 Bispecific CAR lentivirus and (B) Construct diagram showing components of the anti-CD19/CD22 CAR.*

Application

1. Ideal as a positive control for anti-CD19/CD22 Bispecific CAR evaluation in T cells
2. Use in transduction optimization
3. Use to generate anti-CD19/CD22 Bispecific 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

50 µl of anti-CD19/CD22 Bispecific CAR Lentivirus 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 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 protocol described in the "Validation Data" section. Media, reagents, and luciferase assay buffers used at BPS Bioscience 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
Biotinylated Human CD22 Protein	Acrobiosystems #SI2-H82F8-25ug
PE-Streptavidin	Biolegend #405203
CD19/Firefly Luciferase CHO Cell Line	BPS Bioscience #79714
CD22/Firefly Luciferase CHO Cell Line	BPS Bioscience #79715
Firefly Luciferase CHO 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, 1% Penicillin/Streptomycin, plus 10 ng/ml IL-2 (BPS Bioscience #90184).

Experimental Methods and Results:

The following protocol was used to transduce CD4+CD8+ primary T cells with the anti-CD19/CD22 Bispecific 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 StemCell Technologies' instructions. The isolated CD4+ T cells and CD8+ T cells were mixed at a 1:1 ratio and the cells were 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 at 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/CD22 Bispecific 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 freeze/thaw cycles.

   Spinoculation:
   1) 100 µl of T cells (~10,000-20,000 cells) 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 tubes were spun gently at 800 x g for 2 hours at 32°C.

   3) 900 µl of fresh T cell medium was added to 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 ~72 hours. When cell density reached 2 x 10^6 cells/ml, the cells were transferred into a larger cell culture flask with fresh medium.

4. Day 5: The expression of the anti-CD19/CD22 Bispecific CAR was estimated by flow cytometry. The expression of anti-CD19 CAR was analyzed using PE-Labeled anti-FMC63 antibody, and the expression of anti-CD22 CAR was analyzed using biotinylated CD22 followed by PE-Labeled Streptavidin, as shown in Figure 2. The transduced T cells were expanded 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.

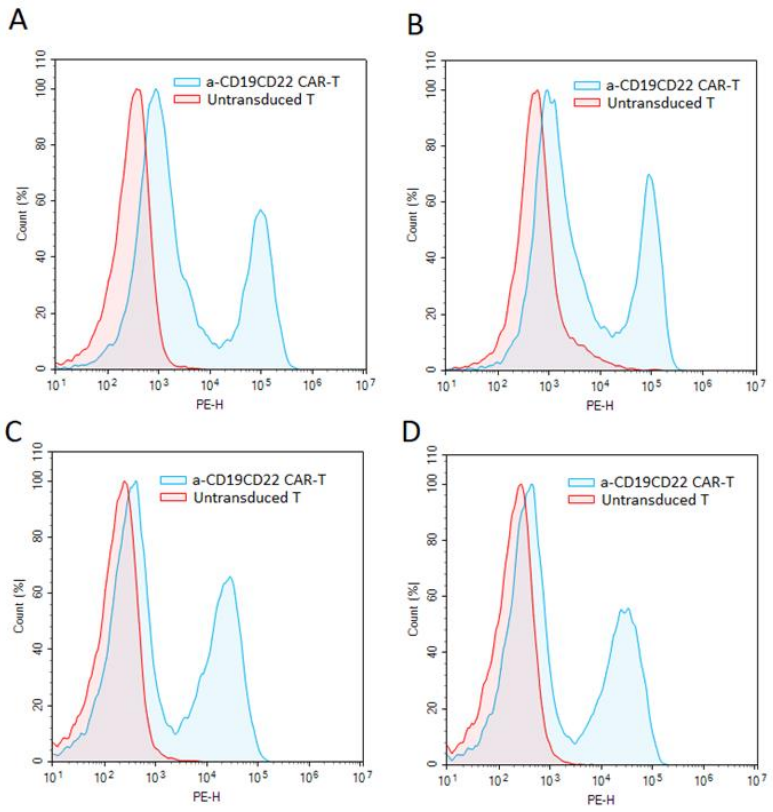


Figure 2. *Expression of anti-CD19/CD22 Bispecific CAR in primary T cells transduced with anti-CD19/CD22 Bispecific CAR lentivirus.*

Approximately 15,000 CD4+CD8+ T cells were transduced with 600,000 TU (at MOI of 40) anti-CD19/CD22 Bispecific CAR Lentivirus in the presence of 5 µg/ml of polybrene via spinoculation. The anti-CD19/CD22 Bispecific CAR expression was analyzed by flow cytometry. The presence of anti-CD19 CAR was detected using PE-anti-FMC63 (Figures 2A and C), and the presence of anti-CD22 CAR was detected using Biotinylated Human CD22 Protein followed with PE-Labeled Streptavidin (Figures 2B and D). A-B, transduced T cells were analyzed 72 hours post-transduction; C-D, transduced T cells were analyzed 10 days post-transduction. Red, Untransduced T cells; Blue, T cells transduced with anti-CD19/CD22 Bispecific CAR lentivirus.

The following experiments are two examples of co-culture assays to evaluate the cytotoxicity of anti-CD19/CD22 CAR-T cells using CD19 or CD22/Firefly Luciferase CHO Cell Lines or Firefly Luciferase Raji Cell Line as the target cells.

Cytotoxicity assay using CD19 or CD22/Firefly Luciferase CHO Cell Line as the target cells

1. Day 10: Target cells CD19/Firefly Luciferase CHO Cell Line (BPS Bioscience #79714) or CD22/Firefly Luciferase CHO Cell Line (BPS Bioscience #79715), which overexpress either CD19 or CD22, 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 or CD22/Firefly Luciferase CHO cells and Firefly Luciferase CHO cells were included for the “no T cell” controls.

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

T cells were centrifuged gently (300 x g for 5 minutes) and resuspended in fresh T cell growth medium. T cells were carefully pipetted into each well at the desired effector:target (E:T) cell ratio in 50 µl of volume. 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. 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.

The plates were incubated at 37°C for 24 hours.

2. Day 11: 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 used for IFNγ expression analysis**.** If the IFNγ assay 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. 50 µl of ONE-Step™ Luciferase assay reagent was added to each well, including medium-only empty wells 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, CD19 or CD22/Luciferase CHO cells was set as 100%. The % Luminescence was calculated as luminescence of co-culture well/ luminescence from the "no T cells" well (Luciferase CHO, CD19 or CD22 Luciferase CHO cells only).

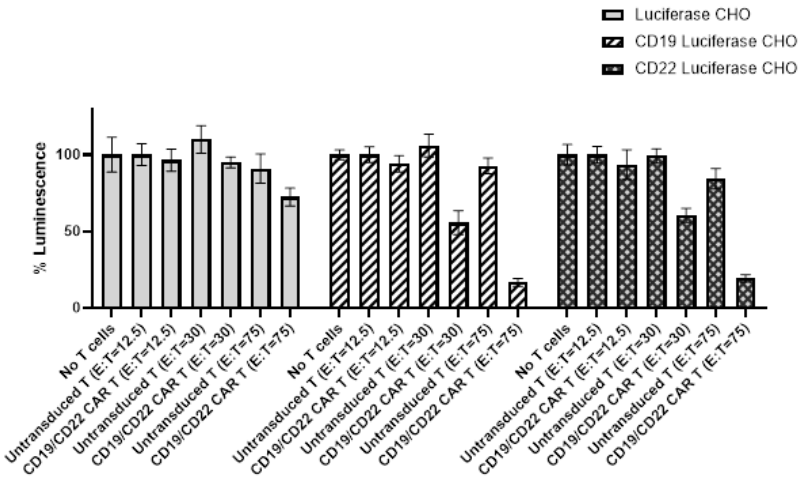


Figure 3. *Luciferase-based cytotoxicity assay using CD19 or CD22/Firefly Luciferase CHO as the target cells.*

Approximately 15,000 CD4+CD8+ T cells were transduced with 600,000 TU (at MOI of 40) anti-CD19/CD22 CAR Lentivirus in the presence of 5 µg/ml of polybrene via spinoculation. Transduced T cells were expanded. Ten days post-transduction, the T cells (effector) were co-cultured with Firefly Luciferase CHO cell, CD19 or CD22/Firefly Luciferase CHO cells (target, seeded at 500 cells/well) for 24 hours at an effector:target ratio of 0, 12.5, 30, and 75. The lysis of target cells was determined by measuring the remaining Luciferase activity. The anti-CD19/CD22 CAR lentivirus transduced T cells showed specific toxicity towards CD19 or CD22/Firefly Luciferase CHO cells. The assay was performed in parallel with untransduced T cells as a negative control.

Cytotoxicity assay using Firefly Luciferase Raji Cells as the target cells

1. Day 10: CD19-CD22-positive target cells "Firefly Luciferase Raji Cell Line" (BPS Bioscience #78622), and negative control cells "Firefly Luciferase K562 Cell Line" (BPS Bioscience #78621) which do not express CD19 or CD22, 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.

    T cells were centrifuged gently (300 x g for 5 minutes) and resuspended in fresh T cell growth medium. T cells were carefully pipetted into each well at the desired effector:target (E:T) cell ratio in 50 µl of volume. 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. Day 11: **Luciferase assay**. The ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was prepared following the recommended protocol. 100 µl of ONE-Step™ Luciferase assay reagent was added to each well and 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 luminescence of co-culture well/ luminescence from the "no T cells" well (Luciferase Raji or Luciferase K562 cells only).

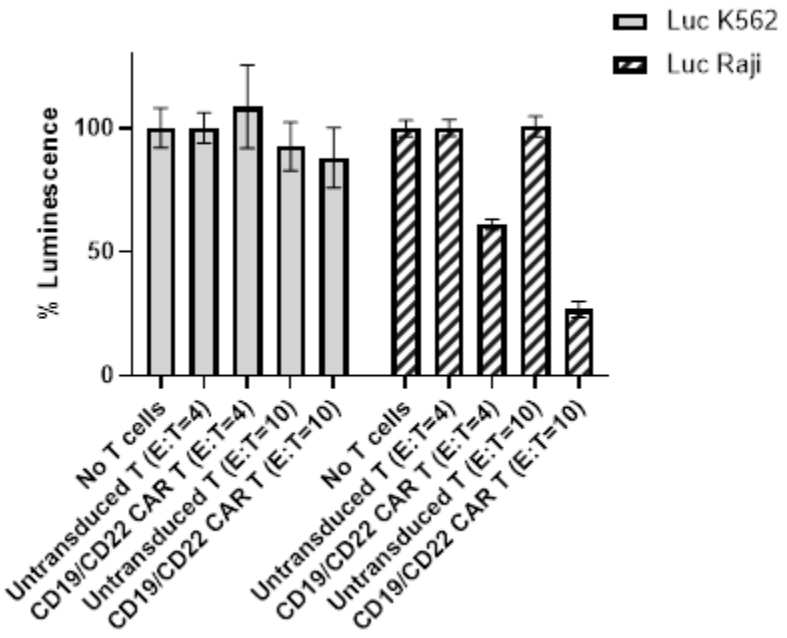


***Figure 4**. Luciferase-based cytotoxicity assay using Firefly Luciferase Raji Cell Line as the target cells.*

Approximately 15,000 CD4+CD8+ T cells were transduced with 600,000 TU (at MOI of 40) anti-CD19/CD22 CAR Lentivirus in the presence of 5 µg/ml of polybrene via spinoculation. Transduced T cells were expanded. Ten days post-transduction, the T cells (effector) were co-cultured with Firefly Luciferase Raji Cells (target, seeded at 5000 cells/well) for 24 hours at indicated effector:target ratio. 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.

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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*
CD19 / Firefly Luciferase - CHO Recombinant Cell Line	79714	2 vials
CD22 / Firefly Luciferase - CHO Recombinant Cell Line	79715	2 vials
Firefly Luciferase - CHO Recombinant Cell Line	79725	2 vials
Firefly Luciferase Raji Cell Line	78622	2 vials
Firefly Luciferase K562 Cell Line	78621	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
ONE-Step™ Luciferase Assay System	60690	10 ml
IFN-γ (Human) Colorimetric ELISA Detection Kit	79777	96 reactions
Human Interleukin-2	90184-A	10 µg
Normal Human Peripheral Blood Mononuclear Cells, Frozen	79059	30 x 10^6 cells