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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
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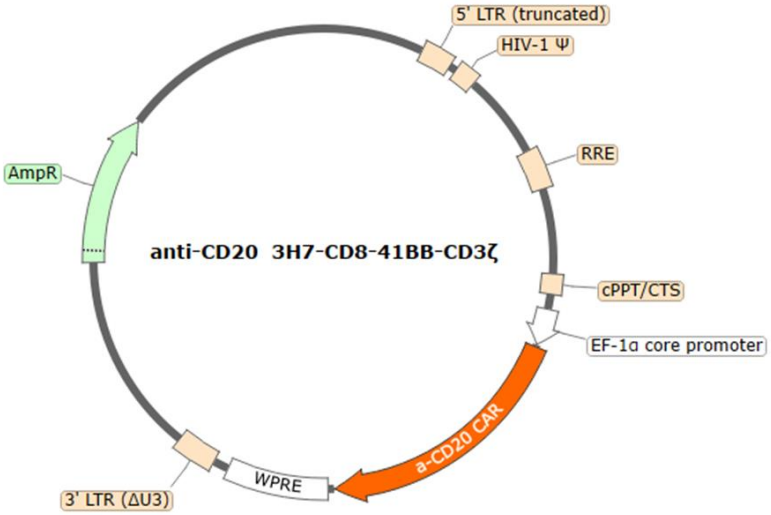
Description

The Anti-CD20 CAR Lentivirus (Clone 3H7-CD8-41BB-CD3ζ) are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce all types of mammalian cells, including primary and non-dividing cells. These viruses transduce the ScFv portion of anti- CD20 (clone 3H7) linked to a 2nd generation CAR (Chimeric Antigen Receptor), containing the CD8 hinge and transmembrane domains, 4-1BB and CD3ζ signaling domains (Figure 1).

These lentiviruses have been validated by flow cytometry (to determine the CAR expression) and in co-culture cytotoxicity assays.

A.



B.

Figure 1: Anti-CD20 CAR Lentivirus (Clone 3H7-CD8-41BB-CD3ζ) construct diagrams.
(A) Schematic of the lenti-vector used to generate the anti-CD20 CAR lentivirus. (B) Construct diagram showing components of the anti-CD20 CAR.

Background

CD20 (also known as MS4A1) is a glycosylated phosphoprotein expressed on the cell surface of B cells. CD20 is a highly attractive target antigen for immunotherapy because it is highly expressed in more than 90% of patients with B-cell lymphoma. First approved in 1997, Rituximab (Rituxan) is a chimeric monoclonal antibody targeting CD20 and has been classified by the World Health Organization as an "Essential Medicine". Since then, additional monoclonal antibodies against CD20 have been approved or are being tested in clinical trials for the treatment of multiple sclerosis (MS), chronic lymphocytic leukemia (CLL), follicular lymphoma, diffuse large B cell lymphoma (DLBCL), rheumatoid arthritis, non-Hodgkin's lymphoma, systemic lupus erythematosus, and myalgic encephalomyelitis (chronic fatigue syndrome). More recently, anti-CD20-CD19 bispecific CAR-T cells have been developed to address concerns over potential relapse in cancer patients.

Application

- Positive control for anti-CD20 CAR evaluation in T cells.
- Transduction optimization experiments.
- Generate anti-CD20 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 upon special request, for an additional fee.

Size and Titer

50 µl of anti-CD20 CAR at a titer ≥ 3 x 10^8 TU/ml. The titer may vary with each lot; the exact value is provided with each shipment.

Storage Conditions

Lentiviruses are shipped with dry ice. For long-term storage, it is recommended to store the virus at -80°C for up to 12 months from the date of receipt.
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 but are necessary for cell culture and for the cellular assays described below. BPS Bioscience's reagents are validated and optimized for use with this product and are highly recommended for the best results.

Name	Ordering Information
PBMC, Frozen	BPS Bioscience #79059
TCellM™	BPS Bioscience #78753
Human Interleukin-2 Recombinant	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
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
Firefly Luciferase - CHO Recombinant Cell Line	BPS Bioscience #79725
CD20/Firefly Luciferase CHO Cell Line	BPS Bioscience #78620
Thaw Medium 3	BPS Bioscience #60186
Biotin-Protein L	Genscript #M00097
PE anti-Biotin Antibody	BioLegend # 409003
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Clear-bottom, white 96-well tissue culture-treated plate	Corning #3610

Media Formulations

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

Assay Protocol

A. Primary T Cell Transduction Protocol

The following protocol was used to transduce $CD4^+$ and $CD8^+$ primary T cells with the Anti-CD20 CAR Lentivirus (Clone 3H7-CD8-41BB-CD3ζ). 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.

Day 0:

1. Isolate $CD4^+$ T cells and $CD8^+$ T cells from previously frozen human PBMC by negative selection, according to manufacturer's instruction.
2. Mix the isolated $CD4^+$ T cells and $CD8^+$ T cells at a 1:1 ratio.
3. Culture the cells using the recommended T Cell Medium at 1 x 10^6 cells/ml density at 37°C with 5% CO_2 overnight.

Day 1:

1. Add T cell activation reagents to the cells according to manufacturer's instruction.
2. Incubate cells at 37°C with 5% CO_2 for 24 - 48 hours.

Day 2:

1. Centrifuge T cells (300 x *g* for 5 minutes) and resuspend in fresh T Cell Medium at 0.1 - 1 x 10^6 cells/ml.

2. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer (5 µg/ml) to the cells.

3. Thaw Anti-CD20 CAR Lentivirus (Clone 3H7-CD8-41BB-CD3ζ) 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:
   1) Distribute 100 µl of T cells (~10,000-100,000) into each 1.5 ml Eppendorf tube.
   2) Add viruses to the cells. Titrate the MOI, starting at an MOI of 10.
   3) Incubate the lentivirus/cells mix in the hood at Room Temperature (RT) for 10 minutes.
   4) Spin the cells/virus mix gently at 800 *x g* for 2 hours at 32°C.
   5) 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 mix.
      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.

      Note: It is not necessary to remove the virus.

5. Incubate the cells at 37°C with 5% CO_2 for ~48-72 hours.

Day 7-14:

1. The expression of the anti-CD20 CAR can be estimated by flow cytometry.

2. Expand cells in T Cell Medium, if desired. These can be frozen in Cryostor® CS10 (BioLife Solutions).

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, to minimize cellular exhaustion. In the experience of scientists at BPS Bioscience, the T cells can expand >1000 fold by 11 days post-transduction, when using TCellM™ supplied with IL-2.

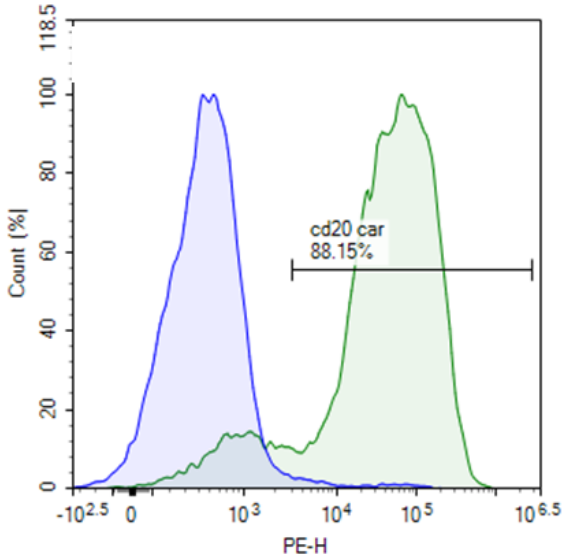


Figure 2. Expression of anti-CD20 CAR in T cells transduced with Anti-CD20 CAR Lentivirus (Clone 3H7-CD8-41BB-CD3ζ).
Approximately 30,000 $CD4^+$ and $CD8^+$ activated T cells were transduced with 600,000 TU (MOI of 20) Anti-CD20 CAR Lentivirus (Clone 3H7-CD8-41BB-CD3ζ) in the presence of 5 µg/ml of Lenti-Fuse™ Polybrene Viral Transduction Enhancer by spinoculation. Anti-CD20 CAR expression was analyzed by flow cytometry 5 days post-transduction (green). Untransduced T cells were used as control (blue). Biotinylated Protein L (Genscript #M00097) and PE anti-Biotin Antibody (BioLegend # 409003) were used for anti-CD20 CAR detection. Y axis represents the % cell number. The X axis indicates fluorophore intensity.

B. Cytotoxicity assay using Firefly Luciferase CHO Recombinant Cell Line and CD20/Firefly Luciferase CHO Cell Line as the target cells.

- The following experiments are an example of co-culture assays to evaluate the cytotoxicity of anti-CD20 CAR-T cells using Firefly Luciferase CHO Recombinant Cell Line and CD20/Firefly Luciferase CHO Cell Line as the target cells.
- This experiment should include "No T Cell Control", "Background Luminescence Control" and "Test Condition".

Day 0:

1. Thaw the frozen anti-CD20 CAR-T (as described above) and control cells in T Cell Medium.
2. Incubate at 37°C for 24 hours.

Day 1:

1. Seed target cells, such as CD20/Firefly Luciferase CHO cells (#79620), and negative control cells, such as Firefly Luciferase - CHO cells (#79725) in 50 µl of Thaw Medium 3 (#60186) at 500 cells/well in a 96-well white, clear bottom tissue culture plate. Leave a few empty wells as "Background Luminescence Control".

2. Centrifuge T cells gently and resuspend in fresh T Cell Medium at the appropriate cell density to reach the desired effector:target (E:T) cell ratio (50 µl/well).

3. Carefully add 50 µl into each well at the desired effector:target (E:T) cell ratio.

4. Add 50 µl of fresh T Cell Medium to the "No T Cell Control" and "Background Luminescence Control" wells. The total volume of each well is 100 µl.

5. Incubate the plates at 37°C for 24 hours.

   Note: No overnight attachment is needed for the adherent target cells. T cells can be added into the wells 1-2 hours after the target cells have been seeded.

Day 2:

1. Add 50 µl of ONE-Step™ Luciferase assay reagent to each well.

2. Incubate the plate at RT for ~15 to 30 minutes and then 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 CHO cells and CD20/Firefly Luciferase CHO cells was set as 100%. The % Luminescence was calculated as background-subtracted luminescence of the co-cultured wells divided by background-subtracted luminescence of the "No T Cell Control" wells (Firefly Luciferase CHO cells and CD20/Firefly Luciferase CHO cells only).

$$\%\ Lum = \frac{Lum\ coculture - background}{Lum\ control - background} * 100$$

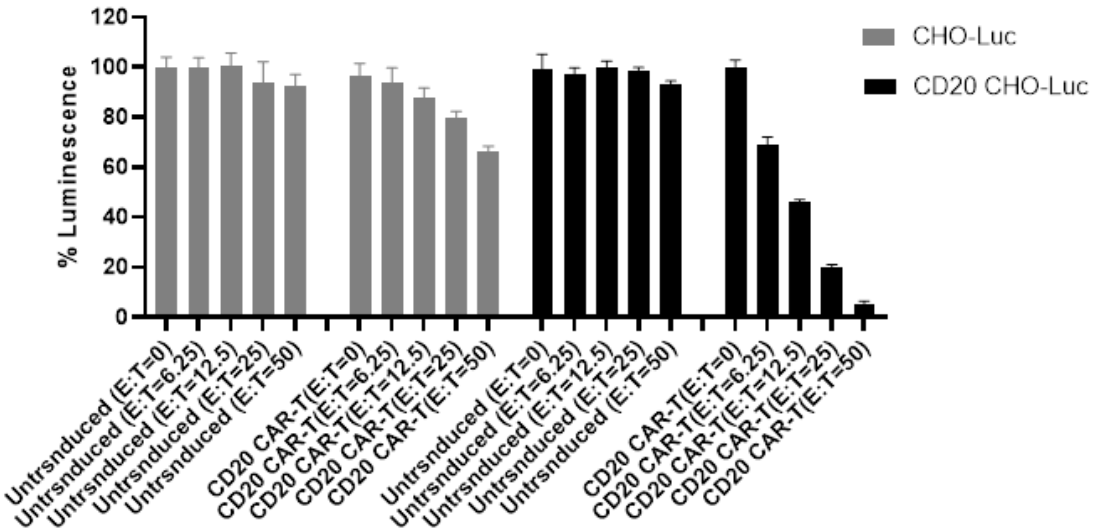


Figure 3. Luciferase-based cytotoxicity assay of T cells transduced with Anti-CD20 CAR Lentivirus (Clone 3H7-CD8-41BB-CD3ζ) using Cell Line and Firefly Luciferase CHO Recombinant Cell Line and CD20/Firefly Luciferase CHO Cell Line as the target cells.
Approximately 30,000 $CD4^+$ and $CD8^+$ T cells were transduced with 600,000 TU (MOI of 20) of Anti-CD20 CAR Lentivirus (Clone 3H7-CD8-41BB-CD3ζ) in the presence of 5 µg/ml of Lenti-Fuse™ Polybrene Viral Transduction Enhancer by spinoculation. The transduced T cells were expanded and frozen on day 12. The thawed T cells (effector) were co-cultured with CD20/Firefly Luciferase CHO cells and Firefly Luciferase CHO cells as the target cells (target) for 24 hours at indicated E:T ratios. The lysis of the target cells was determined by measuring luciferase activity with ONE-Step™ Luciferase Assay System. The assay was performed in parallel with untransduced T cells as a negative control.

Data shown is representative.

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Troubleshooting Guide
Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
Untransduced T cells (Negative Control for CAR-T Cells)	78170	1 vial
Anti-CD20 CAR-T Cells	82491	1 vial
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)	78655	50 µl

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