SZABO SCANDIC

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# Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
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## Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

## Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand


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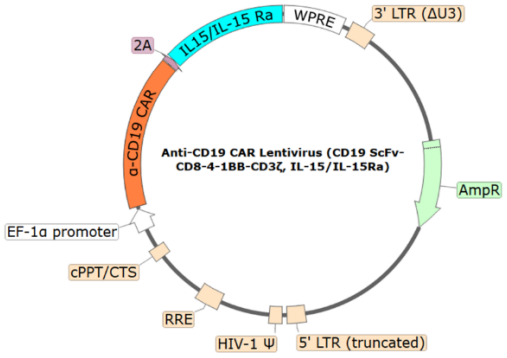
**Description**

The Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ, IL-15/IL-15Ra) are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce 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 a 4$^{th}$ generation CAR (Chimeric Antigen Receptor) containing the CD8 hinge and transmembrane domains, 4-1BB and CD3ζ signaling domains. This construct also includes a 2A-IL15/IL-15 receptor alpha (IL15Ra) sequence downstream of the anti-CD19 CAR cassette to create armored CAR-T cells by overexpressing IL-I5 and IL-15Ra. (Figure 1).

**Note**: This product transduces the same construct as the anti-CD19 CAR Lentiviruses (CD19 ScFv-CD8-4-1BB-CD3ζ) (#78600, 78601, 78602, 78775), but differs in a key aspect:
82378, described here, is constructed with an IL15/IL-15 receptor alpha (IL15Ra) sequence downstream of the anti-CD19 CAR cassette.

| Cat # | Self-Inactivation (SIN) | Selection Marker | Armored with | Costimulatory domain |
|---|---|---|---|---|
| 82378 | yes | no | IL-15 | 41BB |
| 82379 | yes | no | IL-15 | CD28 |
| 78775 | yes | eGFP | N/A | 41BB |
| 78602 | yes | puromycin | N/A | 41BB |
| 78601 | Yes | no | N/A | 41BB |
| 78600 | no | puromycin | N/A | 41BB |

**A.**




**B.**

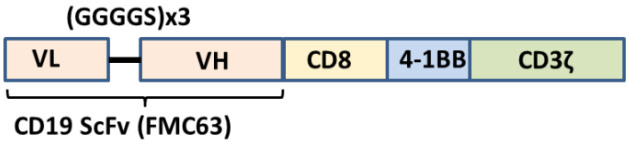



*Figure 1. Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ, IL-15/IL-15Ra) construct diagrams.*
(A) Schematic of the lenti-vector used to generate the anti-CD19 CAR lentivirus. The vector is a SIN vector, and it contains IL15/IL-15 receptor alpha (IL15Ra) sequence downstream of the anti-CD19 CAR cassette. (B) Construct diagram showing components of the anti-CD19 CAR.

**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. Recently, a new generation of CAR-T cells has been developed. These 4$^{th}$ generation CAR-T constructs include a ligand or cytokine, with the goal of armoring the T cells against the TME (tumor microenvironment). The addition of IL-15 has been shown to increase the cytotoxic potential of CAR-T cells both *in vitro* and *in vivo*. The continuous improvement of CAR-T cells will lead to more efficacious cancer therapies. Moreover, it has been reported that engineering NK cells with the IL-15/IL-15R can further improve NK cell proliferation, activation, and cytotoxic activity against B-cell leukemiaα.

**Application**

- Positive control for anti-CD19 CAR evaluation in T/NKcells.
- Transduction optimization experiments.
- Generate anti-CD19 armored CAR-T/CAR-NK 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 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 for up to 12 months from 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 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 |
| 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 |
| PE-Labeled Monoclonal Anti-FMC63 scFv Antibody, Mouse IgG1 (Y45) | Acrobiosystems # FM3-HPY53-25tests |
| IL15 Monoclonal Antibody (34559), PE | ThermoFisher #MA5-23561 |
| CD215 (IL-15Ra) Monoclonal Antibody (eBioJM7A4), PE, eBioscience™ | ThermoFisher #12-7159-42 |
| Firefly Luciferase CD19 Knockout Raji Cell Line | BPS Bioscience #82167 |
| Firefly Luciferase Raji Cell Line | BPS Bioscience #78622 |
| Thaw Medium 2 | BPS Bioscience #60184 |
| Human Peripheral Blood NK Cells, Frozen | BPS Bioscience #78798 |
| Growth-Arrested NK Feeder Cells | BPS Bioscience #78912 |
| NK Medium, Serum-Free | BPS Bioscience #82615 |
| NK Cell Culture Cytokine Cocktail | BPS Bioscience #82616 |
| NK Viral Transduction Enhancer | BPS Bioscience #82617 |
| eGFP/Firefly Luciferase K562 Cell Line | BPS Bioscience #78911 |
| eGFP/Firefly Luciferase RS4; 11 Cell Line | BPS Bioscience #78926 |
| ONE-Step™ Luciferase Assay System | BPS Bioscience #60690 |
| Clear-bottom, white 96-well tissue culture-treated plate | Corning #3610 |

**Media Required for T/NK Cell Culture:**

T Cell Medium: TCellM™ (#78753) plus 10 ng/ml Recombinant Human Interleukin-2 (#90184)

Complete CAR-NK Cell Medium: NK Medium, Serum-Free (#82615) plus 1x NK Cell Culture Cytokine Cocktail (#82616)

**Assay Protocol in primary T cells**

**A. Primary T cell Transduction Protocol**

The following protocol was used to transduce $CD4^+$ and $CD8^+$ primary T cells with Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ, IL-15/IL-15Ra). 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 by negative selection from previously frozen human PBMC, according to the manufacturer's instructions.

2. Mix the isolated $CD4^+$ T cells and $CD8^+$ T cells at a 1:1 ratio.

3. Culture cells using the recommended T Cell Medium at 1 x $10^6$ cells/ml at 37°C with 5% $CO_2$ overnight.

**Day 1:**

1. Add T cell activation reagents to the cells according to the manufacturer's instructions.

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

3. Thaw Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ, IL-15/IL-15Ra) 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 cells) into each 1.5 ml Eppendorf tube.
   2) Add viruses to the cells. Titrate the viral MOI, starting at a MOI of 10.
   3) Incubate the lentiviruses/cells mix in a hood at Room Temperature (RT) for 10 minutes.
   4) Spin the he lentiviruses/cells 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 to each well of a 24-well plate followed by the lentiviruses/cells mix.

      If using 100,000 cells, add 3 ml of fresh T Cell Medium to each well of a 6-well plate followed by the lentiviruses/cells mix.

   *Note: It was not necessary to remove the virus.*

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

**Day 7:**

1. The expression of the anti-CD19 CAR and IL-15/IL-15Ra can be estimated by flow cytometry, using PE-Labeled Monoclonal Anti-FMC63 scFv Antibody, Mouse IgG1 (Y45) to detect anti-CD19 CAR, as shown in Figure 2.

2. The transduced T cells can be further expanded in T Cell Medium.

   *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, T cells can expand >1000 fold by day 11 post-transduction when using TCellM™ supplied with IL-2.*

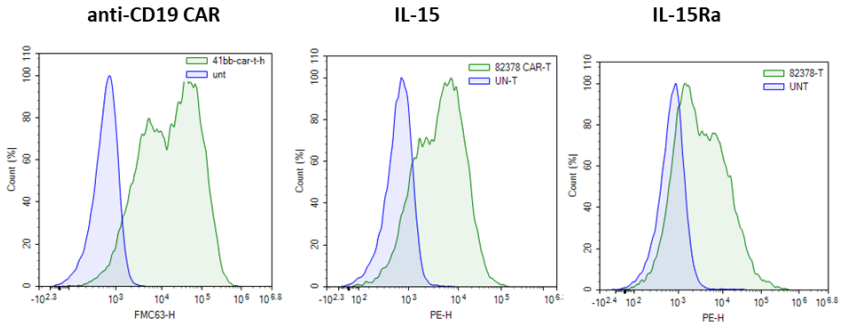



*Figure 2. Expression of anti-CD19 CAR and IL-15/IL-15Ra in T cells transduced with Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ, IL-15/IL-15Ra).*
Approximately 15,000 $CD4^+$and $CD8^+$ activated T cells were transduced with 600,000 TU (MOI of 20) of Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ, IL-15/IL-15Ra) in the presence of 5 µg/ml of Lenti-Fuse™ Polybrene Viral Transduction Enhancer by spinoculation. Anti-CD19 CAR (left panel), IL-15 (middle panel) and IL-15Ra (right panel) expression was analyzed by flow cytometry 5 days post-transduction (green). Non-transduced cells were used as control (blue). Anti-CD19 CAR was evaluated with PE-Labeled Monoclonal Anti-FMC63 scFv Antibody, Mouse IgG1 (Y45) (Acrobiosystems #FM3-HPY53-25tests). IL15 Monoclonal Antibody (34559), PE (ThermoFisher #MA5-23561) and CD215 (IL-15Ra) Monoclonal Antibody (eBioJM7A4), PE, eBioscience™ (ThermoFisher #12-7159-42) antibodies were used for IL-15, and IL-15Ra detection, respectively. Y axis represents the % cell number. The X axis indicates the fluorophore intensity.

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

- The following experiment is an example of a co-culture assay to evaluate the cytotoxicity of anti-CD19 CAR-T using Firefly Luciferase Raji Cell Line as the target cells.
- This experiment should include “No T Cell Control”, “Background Luminescence Control” and “Test Condition”.
- We recommend using Firefly Luciferase CD19 Knockout Raji Cell Line (#82167) as control.

**Day 8:**

1. Seed Firefly Luciferase Raji cells (#78622), and negative control Firefly Luciferase CD19 Knockout Raji cells (#82167) in 50 µl of Thaw Medium 2 (#60184) at 5000 cells/well in a 96-well white, clear bottom tissue culture plate. Prepare a few wells with medium only as “Background Luminescence Control” wells.

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. Add 50 µl of T cells carefully into each well at the desired effector:target (E:T) cell.

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

5. Incubate at 37°C for 24 hours.

**Day 9:**

1. Add 100 µl of ONE-Step™ Luciferase assay reagent to each well.

2. Incubate 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 Raji cells or Firefly Luciferase CD19 Knockout Raji 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 Cell Control" wells.

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

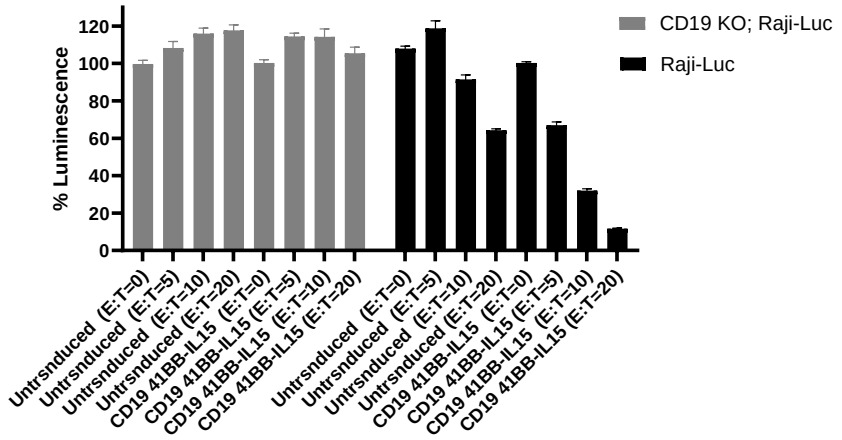



*Figure 3. Luciferase-based cytotoxicity assay of T cells transduced with* Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ, IL-15/IL-15Ra) *using Firefly Luciferase Raji Cell Line as target cells.* Approximately 15,000 $CD4^+$ and $CD8^+$ T cells were transduced with 600,000 TU (MOI of 20) of Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ, IL-15/IL-15Ra) in the presence of 5 µg/ml of Lenti-Fuse™ Polybrene Viral Transduction Enhancer by spinoculation. The transduced T cells were expanded. 7 days post-transduction, T cells (effector cells) were co-cultured with Firefly Luciferase CD19 Knockout Raji cells (CD19 KO; Raji Luc) or Firefly Luciferase Raji cells (Raji-Luc) as the target cells for 24 hours at the indicated E:T ratios. The lysis of the target cells was determined by measuring luciferase activity with ONE-STEP™ Luciferase Assay System. T cells transduced with Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ, IL-15/IL-15Ra) showed specific cytotoxicity towards Firefly Luciferase Raji cells. The assay was performed in parallel with untransduced T cells as a negative control.

### C. CAR-NK Production and Cytotoxicity Assay using eGFP/Firefly Luciferase K562 Cell Line and eGFP/Firefly Luciferase RS4; 11 Cell Line as target cells.

- The following protocol is a general guideline for transducing primary NK cells using Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1-BB-CD3ζ; IL-15/IL-15Ra) (#82378). The optimal transduction conditions (e.g. MOI, time of assay development) may need to be optimized according to the assay requirements.
- Optimal MOI and transduction efficiency of primary NK cells can be donor dependent.
- The following cytotoxicity assay is an example of co-culture assay used to evaluate the cytotoxicity of anti-CD19 CAR-NK cells using eGFP-Firefly Luciferase RS4;11 as target cells. RS4;11, a lymphoblast cell line that expresses HLA-C alleles, that bind the most expressed KIRs (killer-cell immunoglobulin-like receptors), are NK resistant and typically used as negative control in non-transduced NK cytotoxicity assays. However, since they are CD19 positive, RS4;11 cells are good targets for anti-CD19 CAR-NK cells. K562, a human erythromyeloblastoid leukemia cell line, is a NK target due to the lack of HLA expression on the cell surface.
- The assay should include "Background Luminescence Control", "No NK Cell Control" and "Test Condition".
- The assay samples and controls should be run in triplicate.

**Day 1:**

1. Thaw Frozen Human Peripheral Blood NK Cells, (#78798), according to the protocol in the "Cell Thawing and Culture Protocol" section of the datasheet for #78798 using NK Medium, Serum-Free (#82615) supplemented with 1x NK Cell Culture Cytokine Cocktail (#82616). Add Growth-Arrested NK Feeder Cells (#78912) to the NK cells at a ratio of 1:1 and grow the cells in a 5% $CO_2$ incubator at 37°C for 3 days before lentiviral transduction.

**Day 3:**

1. Harvest NK Cells by centrifugation at 300 *x g* for 5 minutes and resuspended in Complete CAR-NK Cell Medium at 0.1-0.2 x $10^6$ cells/ml.

2. Add 1000x NK Viral Transduction Enhancer Components A and B to the cells to have a 1x final concentration of component A and B in the cell suspension and incubate for 30 minutes RT.

3. Transduce the cells with Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ; IL-15/IL-15Ra) (#82378) with the pre-determined optimal MOI in the presence of 1x of NK Viral Transduction Enhancer, by spinoculation at 400 *x g* for 2 hours at 32°C.

4. Transfer the transduced cells to a tissue culture plate, incubate at 37°C with 5% CO2 for 6 hours, remove the virus by refreshing the Complete CAR-NK Cell Medium.

**Day 4:**

1. *Optional: Repeat the lentiviral transduction steps (Day 3 step 1-4).*

2. Culture and expand CAR-NK cells and non-transduced NK cells in Complete CAR-NK Cell Medium.

**Day 7:**

1. Analyze Anti-CD19 CAR expression by flow cytometry.

2. Seed eGFP/Firefly Luciferase K562 and eGFP/Firefly Luciferase RS4; 11 at 5,000 cells/well in 50 µl of Thaw Medium 2 in a 96-well white, clear bottom tissue culture plate. Leave a few empty wells as "Background Luminescence Control" wells.

3. Centrifuge transduced NK cells and control non-transduced NK cells at 300 *x g* for 5 minutes and resuspended the cell pellet in fresh Thaw Medium 2.

4. Determine the desired Effector to Target ratio (E:T) and prepare appropriate cell suspensions (50 µl/well).
5. Carefully pipet 50 µl of NK cell suspension into the appropriate "Test Condition" wells, containing the Firefly Luciferase target cell lines.

6. Add 50 µl of Thaw Medium 2 to the "No NK Cell Control" wells.

7. Add 100 µl of Thaw Medium 2 to the "Background Luminescence Control" wells.

8. Incubate the plates at 37°C with 5% $CO_2$ for 24 hours.

9. Add 100 µl of ONE-Step™ Luciferase assay reagent to each well.

10. Incubate at RT for ~15 to 30 minutes.

**Data Analysis**: the average background luminescence was subtracted from the luminescence reading of all wells. The luciferase activity of Firefly Luciferase target cells was set as 100%. The % Luminescence was calculated as: (luminescence of co-culture well)/(luminescence from the "No NK Cell Control" well). The Specific lysis was calculated as: (1 - % Luminescence Value) x 100.

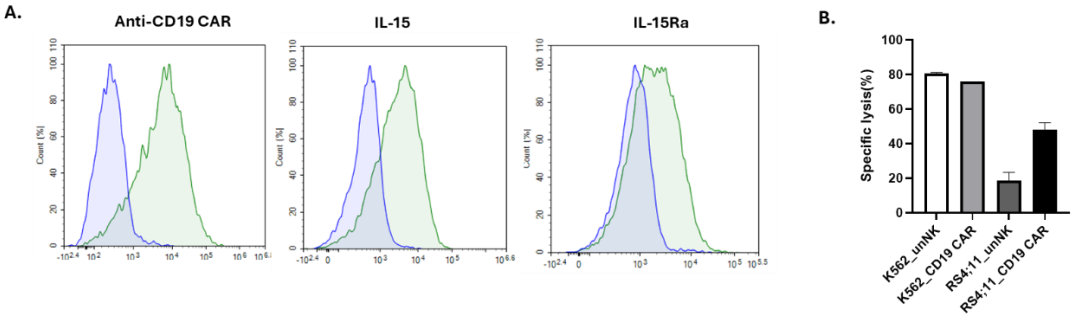



*Figure 3. Expression of anti-CD19 CAR and IL-15/IL-15Ra and luciferase-based cytotoxicity of Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ, IL-15/IL-15Ra) transduced primary NK cells.* Expanded Human Peripheral Blood NK Cells (#78798) were transduced with Anti-CD19 CAR Lentivirus (#82378) at an MOI of 40. Anti-CD19 CAR (left panel), IL-15 (middle panel) and IL-15Ra (right panel) expression was analyzed by flow cytometry 5 days post-transduction (green). Non-transduced cells were used as control (blue). Anti-CD19 CAR was evaluated with PE-Labeled Monoclonal Anti-FMC63 scFv Antibody, Mouse IgG1 (Y45) (Acrobiosystems #FM3-HPY53-25tests). IL15 Monoclonal Antibody (34559), PE (ThermoFisher #MA5-23561) and CD215 (IL-15Ra) Monoclonal Antibody (eBioJM7A4), PE, eBioscience™ (ThermoFisher #12-7159-42) antibodies were used for IL-15, and IL-15Ra detection, respectively. Y axis represents the % cell number. The X axis indicates the fluorophore intensity. B) Transduced NK cells and control non-transduced NK cells were co-cultured with Firefly Luciferase expressing target cells (#78911 and #78926) for 24 hours at an E:T=5 ratio. The lysis of target cells was determined by measuring luciferase activity with ONE-Step™ Luciferase Assay System.

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

**Troubleshooting Guide**

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

**References**

Hawkins E., *et al.*, 2021 *Biologics* 15:95-105.

**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 |

*Version 101624*