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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Primary NK Transduction Kit is a complete kit suitable for primary NK cell engineering using viral transduction. It contains engineered Growth-Arrested NK Feeder Cells (#78912), Frozen Human Peripheral Blood NK Cells (#78798), NK Medium, Serum-Free (#82615), NK Cell Culture Cytokine Cocktail (#82616), and NK Viral Transduction Enhancer (#82617). The kit was optimized using high titer anti-CD19 CAR lentiviruses (such as BPS Bioscience #78601). The kit provides enough components for transduction of a starting population of more than 1 million NK cells.

Background

NK (natural killer) cells are part of the innate immune system. They function in a histocompatibility complex-independent mode and derive from the hematopoietic lineage. They are the first line of defense against cancer. Expression of marker CD56 correlates with NK cell functionality: the CD56bright subset accounts for about 5% of the population and are less cytotoxic than the CD56dim subset. Cytotoxicity can happen by the release of perforin and granzyme, while activation by KARs (killer activating receptors) leads to release of Fas Ligand, TRAIL (TNF-related apoptosis-inducing ligand) and TNFα (tumor necrosis factor-alpha). In a suppressive tumor microenvironment, NK cells can become inhibited and unable to fight cancer cells. Several clinical trials have focused on using *ex vivo* generated NK cells alone or in combination with other approaches. NK cells can be generated *ex vivo* from peripheral blood, umbilical cord blood, iPS cells or immortalized NK cell lines. The ability to generate a number of pure cells high enough for human dosage often requires the use of growth factors such as IL-2 (interleukin 2) or IL-15, and feeder cells.

CAR (chimeric antigen receptor) engineering can add target specific cytotoxicity to NK cells. CAR-NK cells typically have enhanced cytotoxic capacity and cytokine production through co-stimulatory molecules. They may provide stronger tumor-specific targeting and cytotoxicity than CAR-T cells. The use of CAR (chimeric antigen receptor)-NK cells is an expanding area holding great promise in cancer therapy.

Application

Primary NK cell engineering using viral transduction.

Supplied Materials

Catalog #	Name	Amount	Storage
78912	Growth-Arrested NK Feeder Cells	5 million cells	Liquid Nitrogen
78798	Human Peripheral Blood NK Cells, Frozen	1 vial	Liquid Nitrogen
82615	NK Medium, Serum-Free	100 ml	-20°C
82616	NK Cell Culture Cytokine Cocktail	100 µl	-20°C
82617	NK Viral Transduction Enhancer (Components A and B)	100 µl each	-20°C

Storage Conditions

NK Cells and Feeder Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. NK Medium, Serum-Free, NK Cell Culture Cytokine Cocktail, and Viral Transduction Enhancer (Components A and B) are shipped in dry ice and stored at -20°C. After the first thaw, the medium can be stored at 4°C for up to 1 month. Alternatively, aliquot the medium into tubes and store at -20°C. Do not re-freeze aliquots after thawing.

Materials Required but Not Supplied

These materials are not supplied with the Primary NK Transduction Kit but are necessary for CAR-NK characterization and cytotoxicity assays. BPS Bioscience's reagents are validated and optimized for use with this kit and are highly recommended for the best results.

Name	Ordering Information
Anti-NCAM1 Antibody, PE-Labeled	BPS Bioscience #101673
Anti-CD3 Antibody, FITC-Labeled	BPS Bioscience #102008
Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ; SIN Vector)	BPS Bioscience #78601
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
Thaw Medium 2	BPS Bioscience #60184
Clear-bottom, white 96-well tissue culture-treated plate	Corning #3610
Luminometer	

Recommended Complete CAR-NK Medium for CAR-NK generation: NK Medium, Serum-Free (#82615) supplemented with 1x NK Cell Culture Cytokine Cocktail (#82616)

Validation Data

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-CD8-4-1BB-CD3ζ; SIN Vector) (#78601). 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 "Luminescence Background", "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 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 at Room Temperature (RT).

3. Transduce the cells with Anti-CD19 CAR Lentivirus (#78601) 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% CO_2 for 6 hours, remove the virus by refreshing the Complete CAR-NK Medium.

Day 4:

1. Repeat the lentiviral transduction steps (step 1-4 from Day 3).

2. Culture and expand CAR-NK cells and non-transduced NK cells in Complete CAR-NK 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 "Luminescence Background" 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" 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).

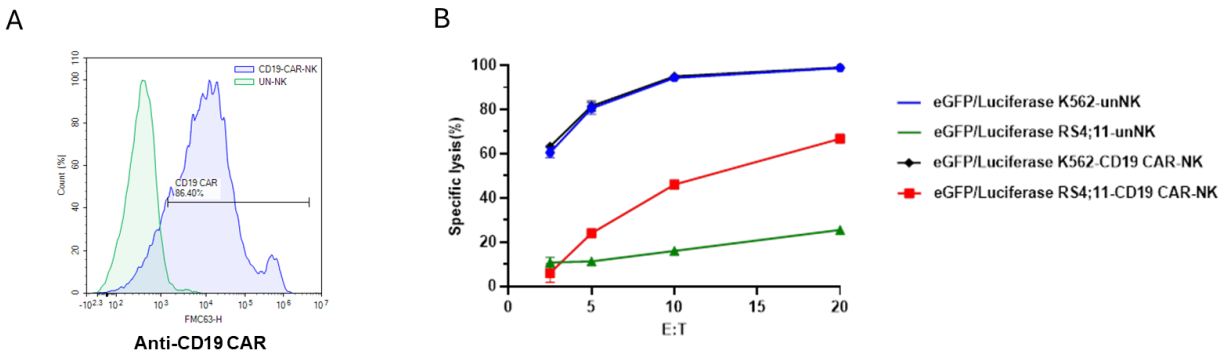

Figure 1. Anti-CD19 CAR-engineered primary NK Cells display both innate cytotoxicity and target specific cytotoxicity.

Expanded Human Peripheral Blood NK Cells (BPS Bioscience #78798) were transduced with Anti-CD19 CAR Lentivirus (BPS Bioscience #78601) at an MOI of 40. A) 72 hours post-transduction Anti-CD19 CAR expression was analyzed by flow cytometry using PE-Labeled Monoclonal Anti-FMC63, Mouse IgG1 (Y45) (Acrobiosystems #FM3-HPY53-25tests). The y axis corresponds to the cell %, while the x axis represents the fluorophore intensity. B) Transduced NK cells and control non-transduced NK cells were co-cultured with Firefly Luciferase expressing target cells (BPS Bioscience #78911 and #78926) for 24 hours at the indicated E:T ratios. 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.

References

Du N., *et al.*, 2021 *Cancers (Basel)* 13 (*16): 4129.*
Liu E., *et al.*, 2020 *N Engl J Med. 382(6):545-553.*

License Disclosure

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Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
NCAM1/CD56 Positive Cell Isolation Kit	78808	1 x 10^8 cells/1x 10^9 cells
NKp46 CHO Cell Line (High, Medium or Low Expression)	78916	2 vials
NKG2D, Avi-Tag, Fc fusion Recombinant	100252	100 µg
NKG2D, Avi-Tag, Fc fusion, Biotin-labeled Recombinant	100313	25 µg/50 µg
NKp46 Lentivirus	78717	500 x 2
Anti-NCAM1 (CD56) IgG Antibody, Biotin-labeled	101112	100 µg

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