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Produktinformation

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

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

Expanded Human Peripheral Blood NK Cells are NK cells enriched and expanded from human PBMCs using the NK Expansion Kit (BPS Bioscience #78927), a K562 feeder cell-based system for about 2-3 weeks, and cryopreserved. Human Peripheral Blood NK Cells are >90 % pure NK cells ($CD3^-CD56^+$ cells), as measured by flow cytometry analysis. They can be used in NK cytotoxicity assays or ADCC (antibody-dependent cellular cytotoxicity), after thaw and recovery in NK Cell Basal Medium for 24 hours or can be further expanded using the BPS Bioscience NK Expansion Kit (BPS Bioscience #78927).

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 CD56 bright subset accounts for about 5% of the population and is 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. The use of NK cells or CAR (chimeric antigen receptor)-NK cells is an expanding area holding great promise in cancer therapy.

Application

- Use in NK cell cytotoxicity assays.
- Use in NK cell mediated ADCC assays.

Materials Provided

Components	Format
5 vial of frozen cells	Vial contains 5 x 10^6 cells in 1 ml of CryoStor® CS10 (Stemcell Technologies #100-1061)

Mycoplasma Testing

The cells have been screened to confirm the absence of Mycoplasma species.

Storage Conditions

NK 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. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Materials Required but Not Supplied

These materials are not supplied with NK cells but necessary for NK characterization and cytotoxicity assays. BPS Bioscience's reagents are validated and optimized for use and are highly recommended for the best results.

Name	Ordering Information
NK Cell Basal Medium	BPS Bioscience #82164
Growth-Arrested NK Feeder Cells	BPS Bioscience #78712
Anti-NCAM1 Antibody, PE-Labeled	BPS Bioscience #101673
Anti-CD3 Antibody, FITC-Labeled	BPS Bioscience #102008
Human Interleukin-2 Recombinant	BPS Bioscience #90184
500 x CFSE	BPS Bioscience #82177
1000 x 7-AAD	BPS Bioscience #82178
K562	ATCC #CCL-243
eGFP/Firefly Luciferase K562 Cell Line	BPS Bioscience #78911
eGFP/Firefly Luciferase RS4; 11 Cell Line	BPS Bioscience #78926
CAR-NK Medium, Serum-Free	BPS Bioscience #82615
NK Cell Culture Cytokine Cocktail	BPS Bioscience #82616
NK Viral Transduction Enhancer	BPS Bioscience #82617
Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ; SIN Vector)	BPS Bioscience #78601
Clear-bottom, white 96-well tissue culture-treated plate	Corning #3610
U-bottomed 96-well plate	Corning #3799
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Thaw Medium 2	BPS Bioscience #60184
Luminometer	

Recommended NK Medium for thawing and expansion: NK Cell Basal Medium (#82164) supplemented with Interleukin-2 (#90184). NK cells can be further expanded using BPS Bioscience feeder cell-based NK Expansion Kit (#78927).

Recommended CAR-NK Medium for CAR-NK generation: For CAR-NK generation we recommend the use of CAR-NK Medium, Serum Free (#82615) with 1x NK Cell Culture Cytokine Cocktail (#82616).

Cell Thawing and Culture Protocol:

- The expansion fold obtained will vary, depending on the source of NK cells and donor.
- It is recommended that cell cultures are monitored daily, and they are split and fed in order to keep optimal cell density (< 2 million/ml).
- Flow cytometry analysis for typical NK markers, such as CD3 and CD56, can be performed to monitor NK purity and determine NK fold expansion.
- Expanded Human Peripheral Blood NK Cells can be expanded further for 3~5 weeks, or other desired expansion period, and then used in downstream applications. Generally, Expanded Human Peripheral Blood NK Cells can reach >90% purity ($CD3^-CD56^+$ cells) and reach >20-fold expansion after further expansion for 1 week.

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed NK Cell Basal Medium.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 20 ml of pre-warmed NK Cell Basal Medium **supplemented with 20 ng/ml Interleukin-2**.

3. Transfer cells into one T75 flask and grow in a 5% CO_2 incubator at 37°C.

4. After 24 hours in a 5% CO_2 incubator at 37°C, NK cells can be directly used in NK cytotoxicity assays or ADCC assays.

   Note: For further expansion follow instructions described below.

5. Add Growth-Arrested NK Feeder Cells to NK cells at a cell-to-cell ratio of 1:1, and grow the cells in a 5% CO_2 incubator at 37°C.

6. Determine cell density every 2-3 days. When the cell density reaches >2 million/ml, dilute cell to 0.25-0.5 x 10^6 cells/mL with NK Cell Basal Medium supplemented with 20 ng/ml IL-2.

7. Incubate the cells at 37°C and 5% CO_2 in a humidified incubator.

8. Refresh medium of NK cells every 2-3 days and refresh feeder cells by providing NK cells with a 1:1 ratio of feeder cells:NK cells weekly.

Validation Data

- The following experiments are an example of co-culture assay used to evaluate the cytotoxicity of NK cells using eGFP-Firefly Luciferase K562 as target cells.
- K562, a human erythromyeloblastoid leukemia cell line, is a NK target due to the lack of HLA expression on the cell surface. 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. RS4;11 cells were used as negative control in the NK cytotoxicity assays.

Luciferase activity-based NK cytotoxicity assay using eGFP/Firefly Luciferase K562 Cell Line as target cells.

- The assay should include a “Minimum Viability Control” (or MIN), “Maximum Viability Control” (or MAX) and “Test” conditions.
- Samples and controls should be run in triplicate.

1. Harvest eGFP-Luciferase K562 cells and seed at 5,000 cells/well in 50 µl of Thaw Medium 2 in a 96-well white, clear bottom tissue culture plate.

2. Prepare Thaw Medium 2 with 2% SDS (50 µl/well of “Minimum Viability Control” wells).

3. Resuspend expanded NK cells in Thaw Medium 2 at the appropriate concentrations to reach the desired Effector:Target (E:T) ratios (50 µl/well).

4. Add 50 µl of NK cells to the target cells by carefully pipetting NK cells to the "Test" wells containing eGFP-Luciferase K562 cells. The total volume of each well is now 100 µl.

5. Add 50 µl of Thaw Medium 2 to the "Maximum Viability Control" wells.

6. Add 50 µl of Thaw Medium 2 with 2% SDS to the "Minimum Viability Control" wells.

7. Incubate the plate at 37°C with 5% CO_2 for 4 hours.

8. Add 100 µl of ONE-Step™ Luciferase reagent.

9. Incubate for 15-30 minutes.

10. Measure luminescence signal in a microplate reader capable of reading luminescence.

Data Analysis: For each target, 3 replicates of the internal references for the 0% viability background (MIN) and the 100% viability maximal signal (MAX) were run.

Percent viability = [(mean luminescence of the experimental sample - mean luminescence of MIN)/(mean luminescence of the MAX - mean luminescence of MIN)] x 100.

Percent Specific Lysis is calculated as follows:
% specific lysis = [1 − (experimental value – MIN value)/ (MAX value – MIN value)] × 100.

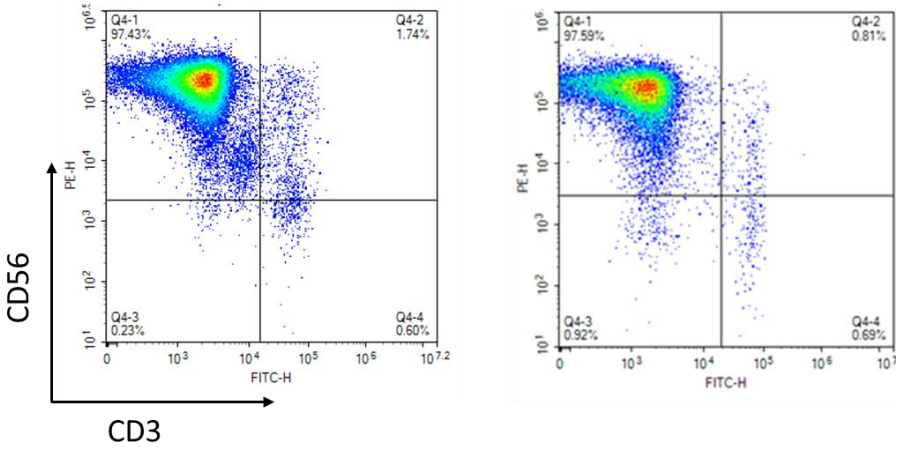

Figure 1. Flow cytometry analysis of Expanded Human Peripheral Blood NK Cells.
Expanded Human Peripheral Blood NK cells were thawed and expanded *ex vivo* for one week (left) and two weeks (right), respectively. Cells were collected and stained with Anti-CD3 Antibody FITC-Labeled and Anti-NCAM-1 Antibody, PE-Labeled and analyzed by flow cytometry.

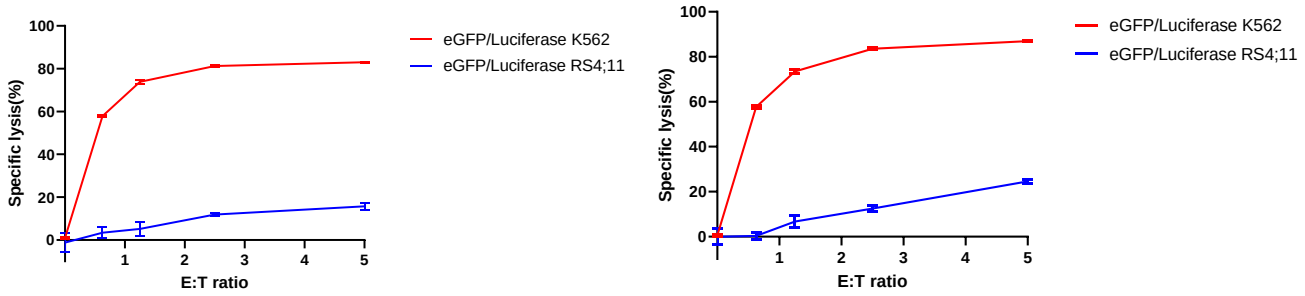

Figure 2. Luciferase-based cytotoxicity profile of Expanded Human Peripheral Blood NK Cells at different time points post-thaw.
Expanded Human Peripheral Blood NK cells cultured for 24 hours post-thaw (left) or a week post-thaw (right) were co-cultured with eGFP/Luciferase K562 target cells and eGFP/Luciferase RS4;11 control cells at indicated ratios of Effector: Target (E:T) cells at 37°C for 4 hours. ONE-Step™ Luciferase Assay was used to detect NK cell cytotoxicity on the luciferase-expressing target cell. Killing curves were obtained based on different Effector: Target (E:T) ratios.

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

- The following protocol is 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 assay should include "Luminescence Background", "No NK Cell Control" and "Test Conditions".
- Samples and controls should be run in triplicate.

Day 1:

1. Thaw frozen NK cells according to the protocol in the "Cell Thawing and Culture Protocol" section using CAR-NK Medium, Serum-Free (#82615) supplemented with 1x NK Cell Culture Cytokine Cocktail (#82616) (Complete CAR-NK Medium). 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 completed CAR-NK Medium, Serum-Free at 0.1-0.2 x 10^6 cells/ml.

2. Add 1000x NK Viral Transduction Enhancer Component 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.

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 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, Serum Free.

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 min 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).

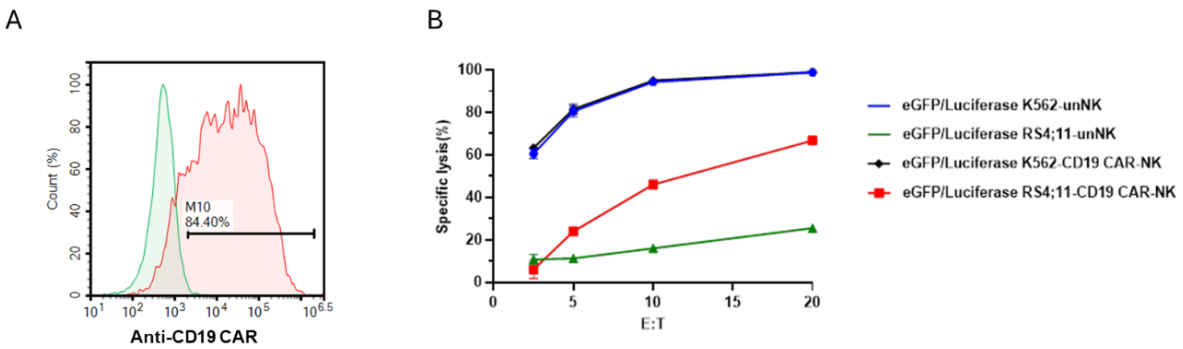

Figure 3. Anti-CD19 CAR-engineered primary NK Cells display both innate cytotoxicity and target specific cytotoxicity.
Expanded Human Peripheral Blood NK Cells (BPS Bioscience# 78798) were transduced with Anti-CD19 CAR Lentivirus (#78601) at a MOI of approximately 40. A) 72 hours post-transduction Anti-CD19 CAR expression was analyzed by flow cytometry using PE-anti-FMC63 ScFv (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.

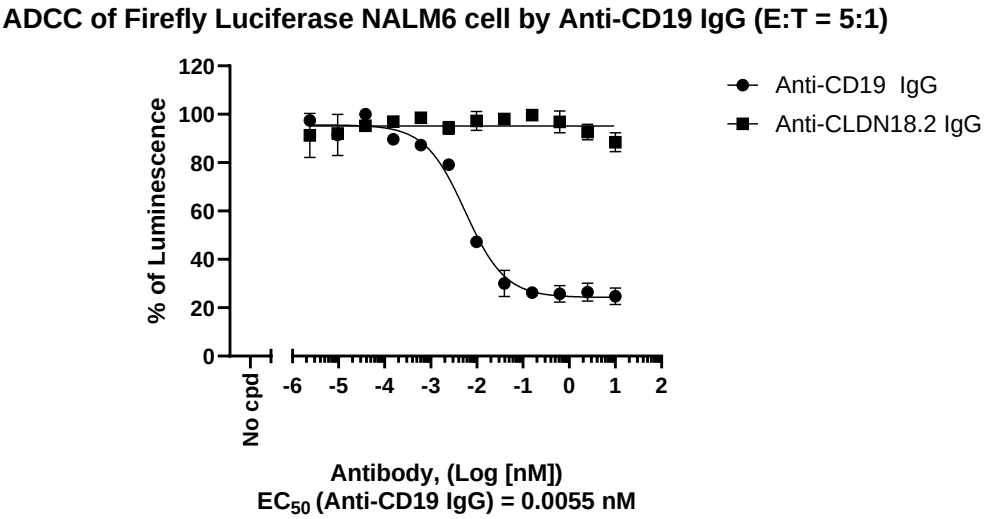

Figure 4. Antibody-dependent cellular cytotoxicity (ADCC) of Firefly Luciferase NALM6 Cell Line triggered by Anti-CD19 IgG.
NK cells and Firefly Luciferase NALM6 cells were combined at a 5:1 ratio in a 96-well white, clear bottom plate. The cells were incubated with a dilution series of Anti-CD19 IgG Antibody (#100981) or the negative control Anti-Claudin-18.2 IgG Antibody (101564). After incubation for 24 hours in a humidified 37°C incubator with 5% CO_2, luciferase activity was measured with One-Step™ Luciferase reagent. For more details, please refer to the database of NK Cytotoxicity Luciferase Assay Kit (NALM6) (#82654). The raw luminescence data were fitted to a sigmoidal three-parameter curve using GraphPad Prism® software.

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.

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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/1 x 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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