Description

Growth-Arrested NK Feeder Cells are suitable for the *ex vivo* culture and expansion of human natural killer (NK) cells. Growth-Arrested NK Feeder Cells are K562 cells engineered to express membrane bound IL-21, and other components, to drive the robust activation and expansion of 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 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

Culture and expansion of freshly isolated or frozen PBMC-derived NK cells, purified and non-purified.

Materials Provided

Components	Format
5 vials of frozen cells	Each vial contains 1 x 10^6 cells

Storage Conditions

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.

Materials Required but Not Supplied

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

Name	Ordering Information
NK Cell Basal Medium	BPS Bioscience #82164
Human Interleukin-2	BPS Bioscience #90184
Human PBMCs	BPS Bioscience #79059
Human Peripheral Blood NK Cells, Frozen	BPS Bioscience #78798
Anti-NCAM1 Antibody, PE-Labeled	BPS Bioscience #101673
Anti-CD3 Antibody, FITC-Labeled	BPS Bioscience #102008
500 x CFSE	BPS Bioscience #82177
1000x 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
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	

NK Expansion Protocol

- The following protocol is a general guideline to expand NK cells from human PBMC, freshly isolated NK cells, or previously frozen NK cells using the Growth-Arrested NK Feeder Cells (alternatively use NK Cell Expansion Kit BPS Bioscience #78927).
- The expansion fold obtained will vary, depending on the source of NK cells and donor.
- The protocol may be adjusted at each step, but we recommend that cells do not reach over 2 million/ml.
- The following instructions are a general guideline for a starter cell number of 1 million (PBMC or isolated NK cells). If more cells are used as starting material, the volume of medium, amount of feeder cells, and culture vessels need to be scaled up accordingly.
- Flow cytometry analysis for typical NK markers, such as CD3 and CD56, can be performed to monitor the NK purity and determine NK fold expansion.

Growth-Arrested NK Feeder Cell Thawing

1. Swirl the vial of frozen feeder cells (2 million/vial) 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 1 ml of pre-warmed NK Cell Basal Medium **supplemented with 20 ng/ml IL-2.**

Co-Culture

1. Prepare PBMCs or NK cells. If using previously frozen PBMCs or NK cells, follow the manufacturer's thawing protocol. Resuspended PBMCs or NK cells in 5 ml of pre-warmed NK Cell Basal Medium **supplemented with 20 ng/ml IL-2.**

2. Add feeder cells to PBMC-derived NK cells as follows (cell/cell ratio). For the starting culture, the total cell number (PBMCs/NK and feeder cells) should be <0.5 million/ml.

   a) Use a 2:1 ratio feeder cells/PBMCs.

   b) Use a 4:1 ratio feeder cells/purified NK cells.

   c) Use a 1:1 ratio feeder cells/BPS Bioscience Human Peripheral Blood NK Cells, Frozen.

   Note: Optimal NK cell to feeder cell ratio may need optimization for your desired culture setup.

3. Grow the cells in a 5% CO_2 incubator at 37°C.

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

5. 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.

   Note: The protocol provided is a general guideline. NK cell growth rates are donor dependent, and subculturing and feeding may need to be performed more frequently. We recommend monitoring NK culture density frequently and keeping it under 2 million/ml. NK cells can be expanded for up to 8 weeks. Generally, if starting from PBMCs, a two-week expansion period can result in >90% NK cell purity and >1,000-fold expansion.

Validation Data

- The following experiments are two examples of co-culture assays used to evaluate the cytotoxicity of NK cells towards A) K562 or B) eGFP-Firefly Luciferase K562 cell lines.
- 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, are NK resistant. RS4;11 cells were used as negative control in the NK cytotoxicity assays.

A. Flow cytometry-based NK cytotoxicity assay using K562 Cell Line as target cells.

1. Harvest K562 cells and stain with CFSE, as described in 500 x CFSE (BPS Bioscience #82177) protocol.

2. Seed CFSE-labeled K562 cells at 50,000 cells/well in 50 µl of Thaw Medium 2, in a U-bottomed 96-well plate.

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 wells containing labeled K562 cells. The total volume of each well is now 100 µl.

5. Incubate plates at 37°C with 5% CO_2 for 4 hours.

6. Spin cells at 300 × *g* for 5 minutes and discard supernatant.

7. Stain with 7-AAD viability dye for 15 minutes in the dark.

8. Analyze the cytotoxicity result by flow cytometry. Gate target K562 cells ($CFSE^+$) and determines the percentage of 7-AAD^+ cells present to calculate NK cell cytotoxicity profiles.

   Note: eGFP-Luciferase K562 Cell Line (BPS Bioscience #78911) can also be used as target cells in a flow cytometry-based NK cytotoxicity assay; target cells ($eGFP^+$) can be gated on the FITC channel. It is necessary to set up the proper instrument gating and compensation adjustments based on the controls when using flow cytometry-based NK cytotoxicity assay.

B. Luciferase activity-based NK cytotoxicity assay using eGFP-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. Measure luciferase activity by adding 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.

Data and Figures

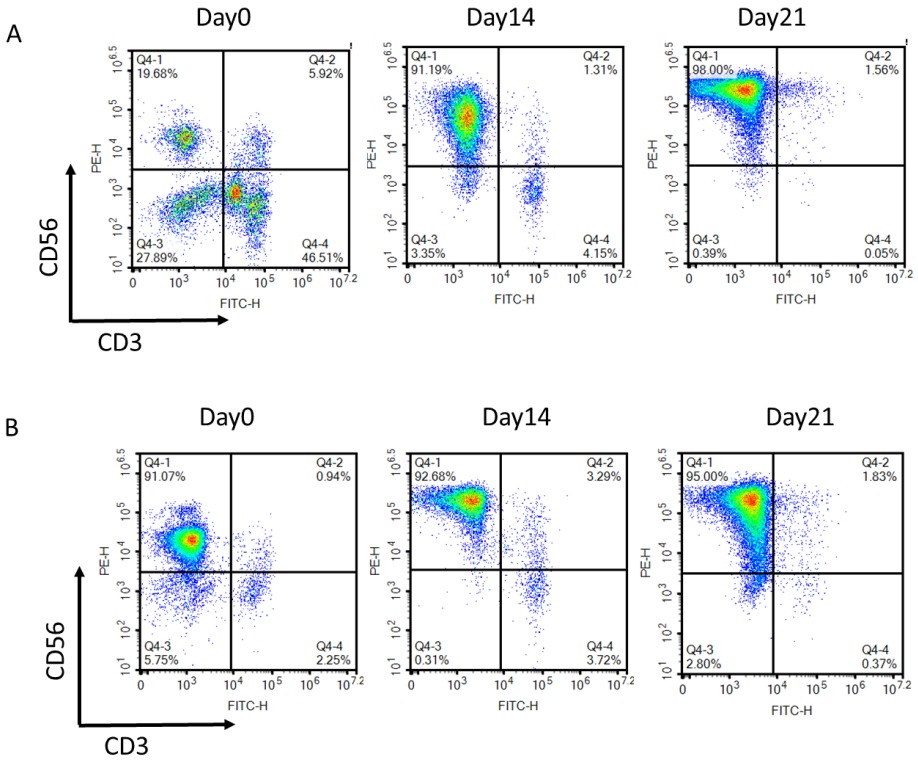


Figure 1. Flow cytometry analysis of NK cell populations at different time points during expansion.
Human PBMCs (A) and isolated NK cells (B) were expanded *ex vivo* for 21 days. Cells were collected on the indicated days of expansion and stained with Anti-NCAM1 Antibody, PE-Labeled and Anti-CD3 Antibody, FITC-Labeled and analyzed by flow cytometry.

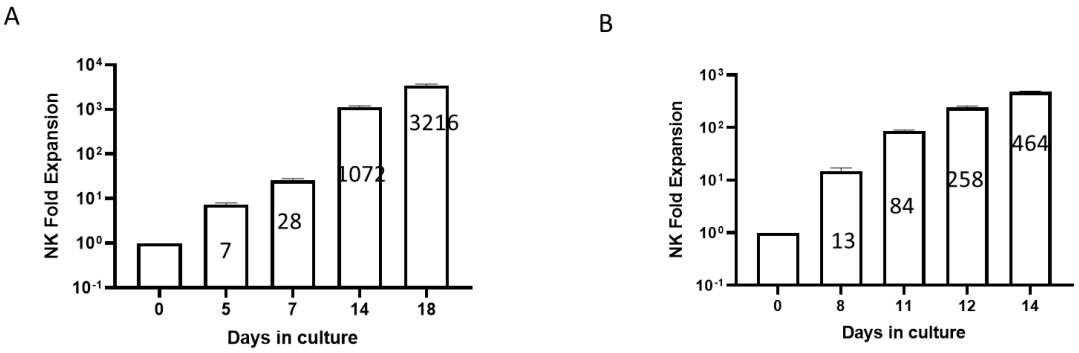


Figure 2. Human NK cell ex vivo fold expansion at different time points during culture.
Human PBMCs (A) and isolated NK cells (B) were expanded *ex vivo* for 14/18 days. Expanded cells were collected on indicated days and stained with anti-NCAM1 Antibody, PE-Labeled and Anti-CD3 Antibody, FITC-Labeled and analyzed by flow cytometry. The number of $CD3^{-}CD56^{+}$ cells was determined, and fold expansion was calculated in relation to $CD3^{-}CD56^{+}$ cell number on day 0.

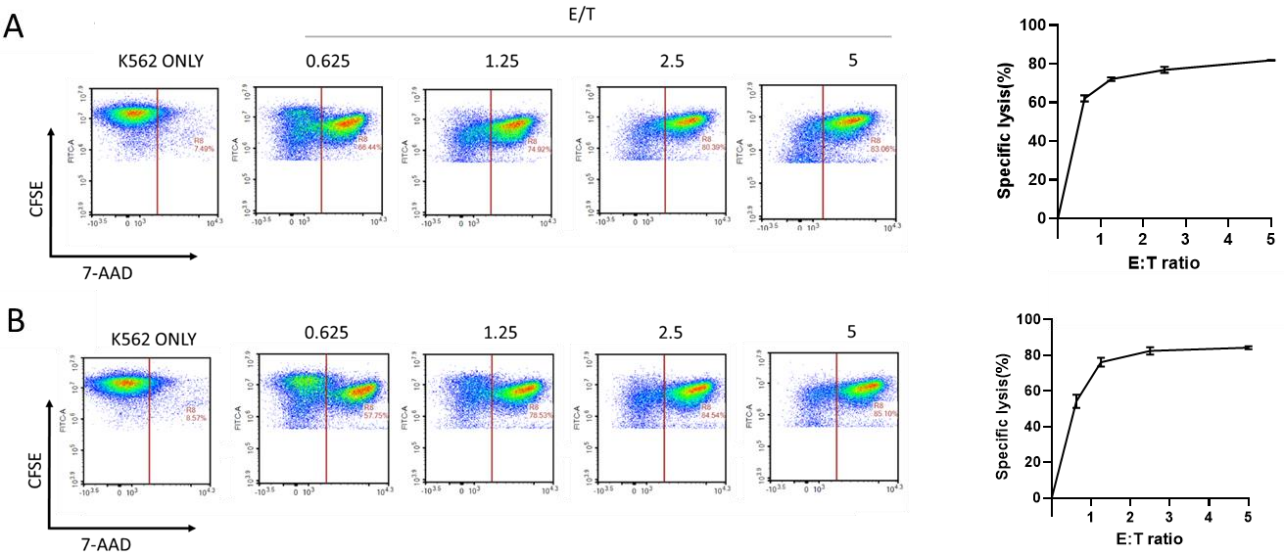
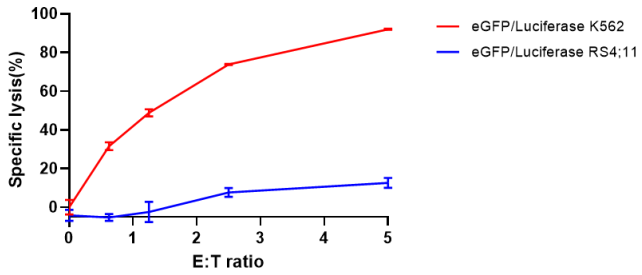


Figure 3. Flow cytometry analysis of the cytotoxicity profile of expanded NK cells.
Human PBMCs (A) and isolated NK cells (B) were expanded *ex vivo* for 14 days. Expanded NK cells were co-cultured with CFSE-labeled K562 target cells at different Effector:Target (E:T) cell ratios at 37°C for 4 hours. 7-AAD was used to detect NK cell cytotoxicity towards K562 cells by flow cytometry (left). Flow cytometry results were used to generate killing curves (right).

A



B

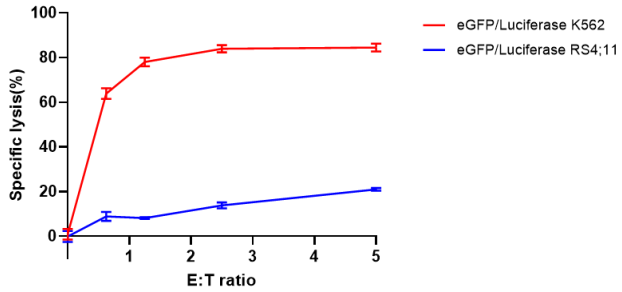


Figure 4. Luciferase activity-based cytotoxicity profile of expanded NK cells.
Human PBMCs (A) and isolated NK cells (B) were expanded *ex vivo* for 14 and 21 days, respectively. Expanded NK cells were co-cultured with eGFP/Luciferase K562 target cells (red) and eGFP/Luciferase RS4;11 control cells (blue) at different Effector: Target (E:T) cell ratios at 37°C for 4 hours. Luciferase activity was detected using ONE-Step™ Luciferase. Cytotoxicity potency (specific lysis) correlates with a decrease in luciferase signal in target cells. Luminescence results were used to generate killing curves.

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.

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

Version 041224