Membrane Bound IL-15 Based Growth-Arrested Feeder Cells

#82374-2

Description

Membrane Bound IL-15 Based Growth-Arrested Feeder Cells are K562 cells engineered to express membrane bound IL-15, and other components, to drive superior functionality of expanded NK cells. They are suitable for the *ex vivo* culture and expansion of human natural killer (NK) cells and γδ (gamma delta) T 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. A recent study indicates that the sequential use of mbIL-21-based followed by mbIL-15-based feeder cells during NK expansion optimizes the yield and function of primary NK cells, this may be the optimal approach in many NK expansion, engineering, and functional study settings. The use of NK cells or CAR (chimeric antigen receptor)-NK cells is an expanding area holding great promise in cancer therapy. The use of feeder cells is also an appropriate method to expand γδ (gamma delta) T cells.

Application

Culture and expansion of primary γδ and NK cells.

Materials Provided

Components	Format
10 vials of frozen cells	Each vial contains 2 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 Medium, Serum-Free	BPS Bioscience #82615
1000x NK Cell Culture Cytokine Cocktail 2	BPS Bioscience #82380
Normal Human Peripheral Blood Mononuclear Cells, Frozen	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
eGFP/Firefly Luciferase K562 Cell Line	BPS Bioscience #78911
eGFP/Firefly Luciferase RS4; 11 Cell Line	BPS Bioscience #78926
Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ; SIN Vector)	BPS Bioscience #78601
PE-Labeled Monoclonal Anti-FMC63 Antibody, Mouse IgG1 (FM3-HPY53)	Acrobiosystems #FM3-HPY53-25tests
Clear-bottom, white 96-well tissue culture-treated plate	Corning #3610
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 PBMCs or previously frozen NK cells using the Membrane Bound IL-15 Based Growth-Arrested Feeder Cells (alternatively use NK Cell Expansion Kit 2, #82393).
- The expansion fold obtained will vary, depending on the source of NK cells and donors.
- 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 2 million PBMC or frozen 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 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 Thaw Medium 2.

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 Medium, Serum-Free (#82615) supplemented with 1x NK Cell Culture Cytokine Cocktail 2 (#82380)**.**

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 Medium, Serum-Free (#82615) supplemented with 1x NK Cell Culture Cytokine Cocktail 2 (#82380).

2. Add feeder cells to PBMC-derived NK cells as follows (cell/cell ratio). For the starting culture, the total cell density (PBMCs/NK and feeder cells) should be at 0.25 - 1.0 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 to 0.25-1.0 x 10^6 cells/ ml with NK Medium, Serum-Free (#82615) supplemented with 1x NK Cell Culture Cytokine Cocktail 2 (#82380).

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 >500-fold expansion.

Validation Data

- The following experiment is an example of a co-culture assay used to evaluate the cytotoxicity of NK cells towards eGFP/Firefly Luciferase K562 cell lines.
- K562, a human erythromyeloblastoid leukemia cell line, is an 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. 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.

B. 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 an 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 2 (#82380).

2. Add Growth-Arrested Feeder Cells (#82374) 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 resuspend at 1 x 10^6 cells/ml in NK Medium, Serum-Free (#82615) supplemented with 1x NK Cell Culture Cytokine Cocktail 2 (#82380).

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 by spinoculation at 400 *x g* for 2 hours at 32°C.

4. Incubate at 37°C with 5% CO_2 for 6 hours, remove the virus by refreshing the NK Medium, Serum-Free (#82615) supplemented with 1x NK Cell Culture Cytokine Cocktail 2 (#82380).

Day 4:

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

2. Culture and expand CAR-NK cells and non-transduced NK cells in NK Medium, Serum-Free (#82615) supplemented 1x NK Cell Culture Cytokine Cocktail 2 (#82380), with weekly stimulation using Membrane Bound IL-15 Based NK Feeder cells.

Day 10-21:

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.

11. Measure 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

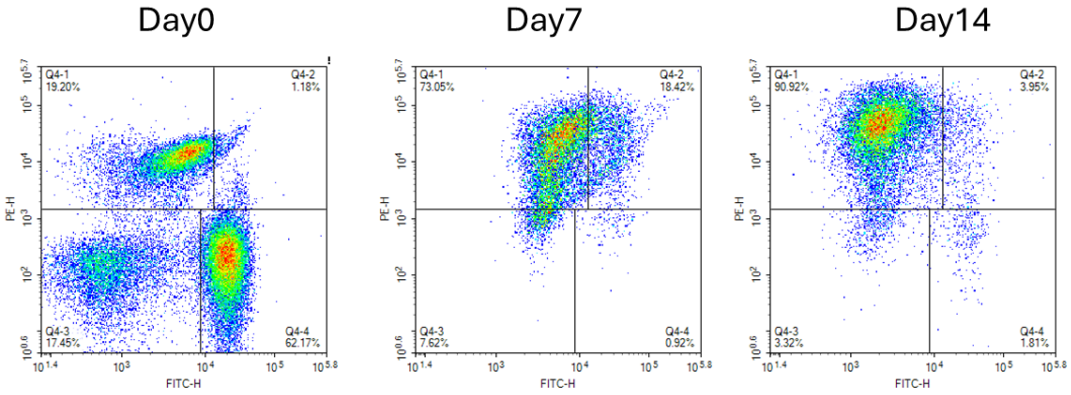

Figure 1. Flow cytometry analysis of NK cell populations at different time points during expansion. Human PBMCs were expanded *ex vivo* for 14 days. Cells were collected on the indicated days of expansion and stained with Anti-NCAM1 Antibody, PE-Labeled and Anti-CD3 Antibody, FITC-Labeled and analyzed by flow cytometry.

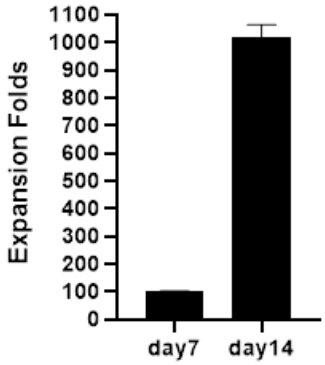

Figure 2. Human NK cell ex vivo fold expansion at different time points during culture.
Human PBMCs were expanded *ex vivo* for 14 days. Expanded cells were collected on indicated days and stained with Anti-NCAM1 Antibody, PE-Labeled and Anti-CD3 Antibody, FITC-Labeled and analyzed by flow cytometry.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

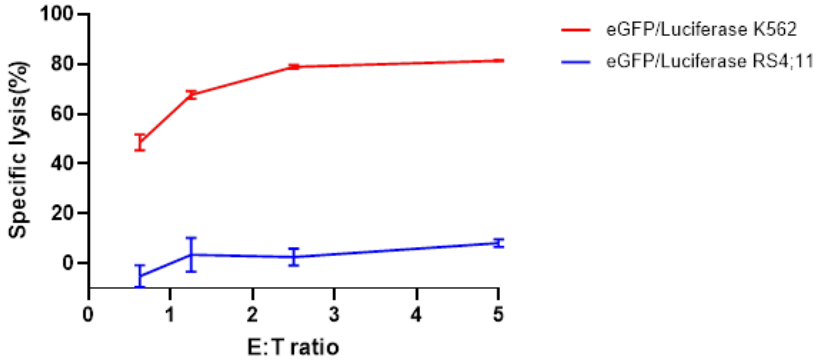

Figure 3. Luciferase activity-based cytotoxicity profile of expanded NK cells.
Human PBMCs were expanded *ex vivo* for 14 days. Expanded NK cells were co-cultured with eGFP/Luciferase K562 target cells (red) and eGFP/Luciferase RS4;11 control cells (blue) at different Effector: Target (E:T) cell ratios 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.

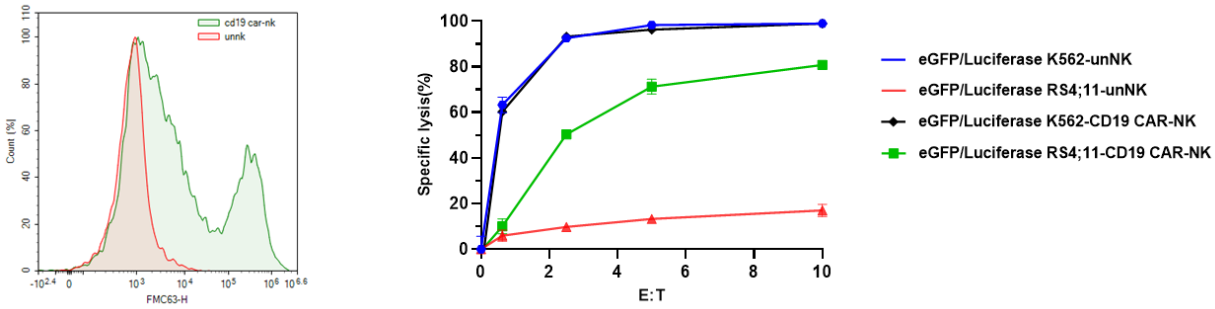

Figure 4. Anti-CD19 CAR-engineered primary NK Cells generated by using Membrane Bound IL-15 Based Growth-Arrested Feeder Cells display both innate cytotoxicity and target specific cytotoxicity.
Expanded Human Peripheral Blood NK Cells (#78798) were cultured with weekly stimulation using Membrane bound IL-15 based Feeder cells. NK cells were transduced with Anti-CD19 CAR Lentivirus (#78601) at an MOI of 40. Left: Anti-CD19 CAR expression was analyzed by flow cytometry 3 weeks post-transduction, 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. Right: 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 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.

References

Du N., *et al.*, 2021 *Cancers (Basel)* 13 (16): 4129.
Zhang C., *et al*., 2023 *Cancer Immunol Res* 11 (11): 1524–1537.

Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
Primary NK Transduction Kit	82618	1 kit
Growth-Arrested NK Feeder Cells	78912	5 x 1m, 5 x 2m, 10 x 2m
NK Cell Basal Medium	8261	100 ml, 500 ml
NK Viral Transduction Enhancer	82617	100 µl
Untransduced NK Cells	82315	1 vial
Anti-CD19 CAR-NK cells; 41BB	82316	1 vial
Anti-CD19 CAR-NK cells; CD28	82386	1 vial

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