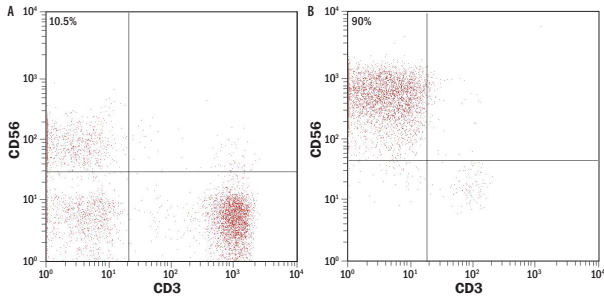


## DATA EXAMPLES



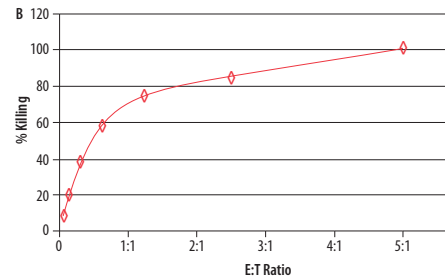
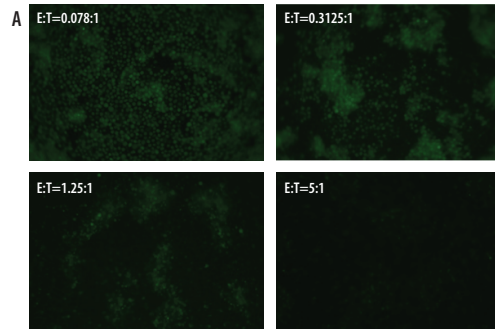
**Figure 1: Flow Cytometry Analysis of Expanded Human NK Cells.** Human PBMCs were expanded *in vitro* for 14 days using reagents included in the CellXVivo™ Human NK Cell Expansion Kit. Compared to unexpanded PBMCs (A), PBMCs treated with the kit (B) show an increased number of CD3<sup>+</sup>CD56<sup>+</sup> NK cells. Flow cytometric analysis was performed on day 14 of the expansion and cells were stained with Human NCAM-1/CD56 PE-conjugated Antibody, and Human CD3ε PerCP-conjugated Antibody. Quadrants were set based on isotope-stained samples. All R&D Systems® antibodies and corresponding catalog numbers used are shown in the table below.

CATALOG #	DESCRIPTION
FAB2408P	Human NCAM-1/CD56 PE-conjugated Antibody
IC0041P	Mouse IgG <sub>2b</sub> PE-conjugated Antibody Isotype Control
FAB100C	Human CD3ε PerCP-conjugated Antibody
IC002C	Mouse IgG <sub>1</sub> PerCP-conjugated Antibody Isotype Control

## PRECAUTION

This product contains human serum from human source material. This human source material was tested at the donor level using an FDA licensed method and found to be non-reactive for anti-HIV-1/2 and Hepatitis B surface antigen. As no testing can offer complete assurance of freedom from infectious agents, these reagents should be handled as if capable of transmitting infection.

## DATA EXAMPLES CONTINUED



**Figure 2: Kit-expanded NK Cells are Cytotoxic to Tumor Cells.**

NK Cells expanded using the CellXVivo™ NK Cell Expansion Kit were assessed for toxicity against the NK cell-sensitive K562 tumor cell line. K562 cells were loaded with the live-cell dye, Calcein-AM (Tocris, Catalog # 5119) prior to being mixed with NK cells for 4 hours at the indicated effector-to-target cell (E:T) ratios (A). (B) Graph showing % Killing of tumor cells by NK cells at the indicated E:T ratios.

## REFERENCES

1. Caligiuri, M.A. (2008) *Blood* **112**:461.
2. Cooper, M.A. *et al.* (2001) *Blood* **97**:3146.
3. De Maria, A. *et al.* (2011) *Proc. Natl. Acad. Sci. U S A* **108**:728.
4. Vivier, E. *et al.* (2008) *Nat. Immunol.* **9**:503.
5. Chester, C. *et al.* (2015) *Front. Immunol.* **6**:601.

CellXVivo™

## Human NK Cell Expansion Kit

Catalog Number: CDK015

## BACKGROUND

Natural Killer (NK) cells were identified as a distinct sub-population of lymphocytes that have the capacity to spontaneously lyse tumor cells (1). Human NK cells are phenotypically characterized by the expression of CD56 and the absence of CD3 (2). NK cells produce immunoregulatory cytokines, including interferon-γ (IFN-γ), tumor necrosis factor-beta (TNF-β), tumor necrosis factor-α (TNF-α), granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin (IL)-10, and IL-13. Activation of NK cells can be triggered via NKp46 and NKp30 receptors or after stimulation with combinations of IL-2, IL-12, and IL-15 (3). NK cells play an important role in both the adaptive and innate immune responses that govern infection, autoimmunity, and tumor immunosurveillance (4). Preclinical evidence and early clinical success has established NK cell immunotherapy as a promising therapeutic strategy in cancer (5). The CellXVivo™ Human NK Cell Expansion Kit contains base media and NK expander cocktails for the optimized expansion of highly cytotoxic CD3<sup>+</sup>CD56<sup>+</sup>NK cells from peripheral blood mononuclear cells (PBMCs).

This package insert must be read in its entirety before using this product.  
For research use only. Not for use in diagnostic procedures.

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## MATERIALS PROVIDED & STORAGE CONDITIONS

COMPONENTS	PART #	# VIALS	STORAGE OF OPENED/ RECONSTITUTED MATERIAL	SHIPPING CONDITIONS
Human Killer Cell Base Media 2	968386	1 (250 mL)	Store at 2-8 °C under sterile conditions*.	Kool packs**
NK Cell Expander 1	968387	1 vial	Store at 2-8 °C under sterile conditions for up to 30 days or at -20 °C to -70 °C in a manual defrost freezer for up to 3 months.*	Dry ice**
NK Cell Expander 2	968388	1 vial		
NK Cell Expander 3	968389	1 vial		
NK Cell Expander 4	968390	1 vial		
NK Cell Expander 5	968393	1 (25 mL)		
Reconstitution Buffer 1	967552	1 vial		
Reconstitution Buffer 2	967553	1 vial		
20X Wash Buffer	967557	2 vials		

\* Provided this is within the expiration date of the kit.

\*\* The components for this kit require different storage/shipping temperatures and will arrive in separate packaging.

## OTHER MATERIALS & SUPPLIES REQUIRED

- Ficoll-Hypaque™
- 75 cm<sup>2</sup> cell culture flasks
- Microscope
- Hemocytometer
- 37 °C, 5% CO<sub>2</sub> incubator
- Centrifuge
- Pipettes and pipette tips
- Sterile deionized water

## REAGENT PREPARATION

**Wash Buffer (1X)** - Dilute Wash Buffer (20X) 1:20 in sterile deionized water to produce Wash Buffer (1X).

**NK Cell Expander 1 (400X)** - Reconstitute NK Cell Expander 1 with 150 µL of Reconstitution Buffer 2 to produce NK Cell Expander 1 (400X) stock solution.

**NK Cell Expander 2 (1000X)** - Add 250 µL of Reconstitution Buffer 1 to NK Cell Expander 2 to produce NK Cell Expander 2 (1000X).

**NK Cell Expander 3 (1000X)** - Add 250 µL of Reconstitution Buffer 1 to NK Cell Expander 3 to produce NK Cell Expander 3 (1000X).

**NK Cell Expander 4 (1000X)** - Add 250 µL of Reconstitution Buffer 1 to NK Cell Expander 4 to produce NK Cell Expander 4 (1000X).

**NK Cell Expander 5 (10X)** -Thaw NK Cell Expander 5 at 2-8 °C or at room temperature.

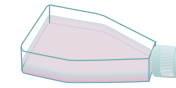
**Human NK Cell Expansion Media** - Add 25 µL of NK Cell Expander 2 (1000X), NK Cell Expander 3 (1000X), NK Cell Expander 4 (1000X), and 2.5 mL of NK Cell Expander 5 (10X) to 22.5 mL of Human Killer Cell Base Media 2 to make 25 mL of Human NK Cell Expansion Media.

## PROTOCOL FOR NK CELL EXPANSION

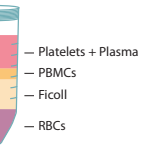
1. Prepare 6 mL of NK Cell Expander 1 (1X) for each 75 cm<sup>2</sup> cell culture flask by diluting the NK Cell Expander 1 (400X) stock solution 1:400 in Wash Buffer (1X).
2. Coat 75 cm<sup>2</sup> cell culture flasks by adding 6 mL of NK Expander 1 (1X) to each flask. Incubate at 2-8 °C overnight. Remove NK Cell Expander 1 (1X) from the flasks before use.
3. Isolate human PBMCs from human blood using Ficoll-Hypaque™ density gradient centrifugation.
4. Perform a cell count and suspend human PBMCs at 1 x 10<sup>6</sup>/mL in Human NK Cell Expansion Media.
5. Add 25 mL of suspended human PBMCs into each pre-coated 75 cm<sup>2</sup> flask. Incubate cells in a 37 °C, 5% CO<sub>2</sub> humidified incubator.
6. On day 4, refresh the Human NK Cell Expansion Media by first harvesting the suspended cells into a 50 mL centrifuge tube and then pelleting the cells by centrifugation at 300 x g for 5 minutes. Resuspend the cell pellets in 25 mL of fresh Human NK Cell Expansion Media and add cell suspension back to the same flask.
7. On day 6, for each 75 cm<sup>2</sup> cell culture flask in use, coat four new 75 cm<sup>2</sup> cell culture flasks with NK Cell Expander 1 (1X) as described in Step 2.
8. On day 7, split the cells 1:4 into the new 75 cm<sup>2</sup> cell culture flasks which were coated with NK Cell Expander 1 (1X). First, harvest the suspended cells into a 50 mL sterile centrifuge tube and then centrifuge at 300 x g for 5 minutes. Resuspend the cell pellets in 40 mL of fresh Human NK Cell Expansion Media. Add 10 mL of the resuspended cells into each of four new 75 cm<sup>2</sup> flasks which were coated with NK Cell Expander 1 (1X). Add 15 mL of fresh Human NK Cell Expansion Media to each flask for a final volume of 25 mL.
9. On day 10, refresh Human NK Cell Expansion Media as described in step 6.
10. On day 14, harvest expanded NK cells. NK cells are ready for downstream applications.
11. **Optional:** To verify NK cell expansion via flow cytometry, collect the cells and wash with PBS once. Process, stain, and analyze to determine CD3 and CD56 expression on the cell surface. Analyze marker expression via flow cytometry as shown in the Data Examples.

## PROTOCOL OUTLINE

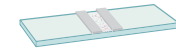
**Coat** 75 cm<sup>2</sup> flask with NK Cell Expander 1 (1X).



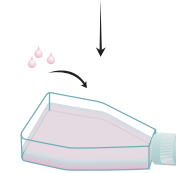
**Isolate** PBMCs from human blood.



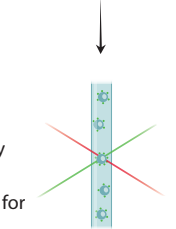
**Perform** a cell count.



**Suspend** 1 x 10<sup>6</sup> PBMCs/mL in Human NK Cell Expansion Media. **Culture** the cells in the pre-coated 75 cm<sup>2</sup> flask for a total of 14 days.



**Verify** expanded NK cells by analyzing NK cell marker expression via flow cytometry (**optional**).



**NK cells** are ready to be used for downstream applications.