Cloudz™ Human NK Cell Expansion Kit

Catalog Number CLD004

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

TABLE OF CONTENTS

Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956
FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

DESCRIPTION

The Cloudz Human NK Cell Expansion Kit is designed for the robust expansion of human Natural Killer (NK) cells from peripheral blood mononuclear cells (PBMCs) using a feeder-free culture system. The kit contains dissolvable microspheres that are functionalized with anti-CD2 and anti-NKp46 antibodies (Cloudz CD2/NKp46). The kit also contains 6X Release Buffer which enables easy cell harvesting by quickly dissolving Cloudz CD2/NKp46. Each kit contains enough materials to activate and expand 50 x 10^6 NK cells from PBMCs, with greater than 150-fold expansion in 14 days.

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-β (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). Published preclinical and clinical data has established NK cell immunotherapy as a promising therapeutic strategy in cancer (5). Continued in vitro research on methods for NK cell expansion, for monitoring NK cell targeting kinetics and for understanding mechanisms of cytotoxicity, are necessary to elucidate NK cell needs in both preclinical and therapeutic settings.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- Do not use if package is damaged. Use undamaged and sealed bottles only.
- This reagent should not be used beyond the expiration date indicated on the label.
- Results may vary due to variations among primary NK lymphocyte populations derived from different donors.
- The most recent version of the End User Terms of Use of Product may be found at: RnDSystems.com/Legal-information.

PRECAUTIONS

When handling bio-hazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	AMOUNT PROVIDED	STORAGE OF OPENED/RECONSTITUTED MATERIAL
Cloudz CD2/NKp46	SPC-4231	9 mL	Store at 2-8 °C.*
6X Release Buffer	SPC-4262	100 mL	

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

OTHER MATERIALS REQUIRED

- Recombinant Human IL-2 (R&D Systems®, Catalog # 202-IL)
- Recombinant Human IL-12 (R&D Systems, Catalog # 219-IL)
- Recombinant Human IL-18 (R&D Systems, Catalog # 9124-IL)
- Recombinant Human IL-21 (R&D Systems, Catalog # 8879-IL)
- Media*:
  ExCellerate™ Human NK Cell Expansion Media, Xeno-Free (R&D Systems, Catalog # CCM032)
- Sterile Phosphate Buffered Saline (PBS)
- Pipettes and pipette tips
- 15 mL and 50 mL Polypropylene Centrifuge Tubes
- 24-well tissue culture plate
- 25 cm^2 tissue culture flask (T25)
- 75 cm^2 tissue culture flask (T75)
- 37 °C, 5% CO_2 incubator
- Inverted microscope
- Flow Cytometer
- Cell counting materials
- Centrifuge

*If using the alternate serum procedure, use Fetal Bovine Serum - Premium, R&D Systems, Catalog # S11150 or equivalent.

RECOMMENDED PROCEDURE:
EX VIVO CULTURE OF HUMAN NK CELLS USING SERUM-FREE CONDITIONS

REAGENT PREPARATION

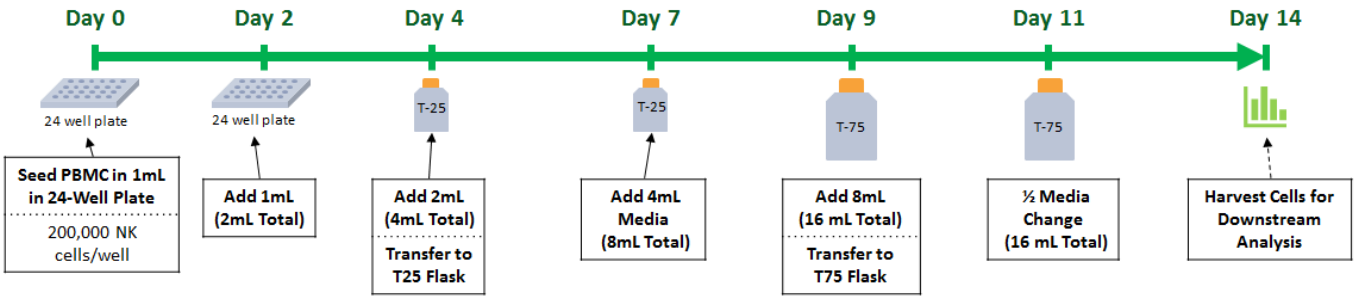
1X Complete ExCellerate Human NK Cell Expansion Media - Determine the amount of media needed for your experiment (*i.e.* 1 mL of media is needed per well of a 24-well plate). Immediately before use, add the following cytokines with the following recommended final concentrations: Recombinant Human IL-2 (27 ng/mL), Recombinant Human IL-12 (10 ng/mL), Recombinant Human IL-18 (10 ng/mL), and Recombinant Human IL-21 (10 ng/mL).

2X Complete ExCellerate Human NK Cell Expansion Media - Determine the amount of media needed for your experiment. Immediately before use, add the following cytokines with the following recommended final concentrations: Recombinant Human IL-2 (54 ng/mL), Recombinant Human IL-12 (20 ng/mL), Recombinant Human IL-18 (20 ng/mL), and Recombinant Human IL-21 (20 ng/mL).

1X Release Buffer - Immediately before use, dilute 6X Release Buffer 1:6 using Sterile 1X PBS to produce 1X Release Buffer. (*i.e.*, add 10 mL of 6X Release Buffer to 50 mL of Sterile 1X PBS to generate 1X Release Buffer).

RECOMMENDED PROTOCOL

The protocol below describes the expansion of human NK cells using ExCellerate™ Human NK Cell Expansion Media, Xeno-Free (R&D Systems®, Catalog # CCM032) in combination with the Cloudz™ Human NK Cell Expansion Kit (R&D Systems®, Catalog # CLD004) and recombinant cytokines. This protocol starts in a 24-well plate and expands to larger cell culture vessels to accommodate NK cell expansion. NK cell expansion may vary by donor.



- This protocol was developed to accommodate most donors; however, there is a large amount of donor variation. Results may be improved by more frequent media changes as needed, most likely during the largest growth phase between days 6 and 14.
- Different optimized combinations of recombinant cytokines, including IL-2, IL-12, IL-18, and IL-21 can be used. The activation and cytokine/growth factor combinations used with this media should be optimized by application or experimental protocol.

Day 0

1. Pre-warm the required amount of 1X Complete ExCellerate™ Human NK Cell Expansion Media to room temperature.
2. Isolate peripheral blood mononuclear cells (PBMCs) or $CD3^+$-depleted PBMCs using desired protocol. Determine the concentration (NK cells/mL) in the starting cell population using flow cytometry. For most donors, NK cells account for 5-10% of PBMCs.
3. Dilute cell suspension to approximately 0.2 x 10^6 NK cells/mL in 1X Complete ExCellerate Human NK Cell Expansion Media.
4. Seed the starting NK cell culture by adding 1 mL/well (0.2 x 10^6 NK cells) of the cell suspension to a desired number of wells of a 24-well plate.
5. Add 30 µL Cloudz CD2/NKp46 (Cloudz Human NK Cell Expansion Kit) to each well
6. Incubate at 37 °C and 5% CO_2 in a humidified incubator.

Day 2

7. Add 1 mL per well of pre-warmed 2X Complete ExCellerate Human NK Cell Expansion Media. Total media should be 2 mL/well. Incubate at 37 °C and 5% CO_2 in a humidified incubator.

Day 4

8. Transfer the cell suspension (including Cloudz CD2/NKp46) from one well of a 24-well plate into one T25 flask. Add 2 mL of pre-warmed 2X Complete ExCellerate Human NK Expansion Media. Total media should be 4 mL/flask. Repeat for each additional well of the 24-well plate. Place flasks vertically (vented flask cap facing up) in a humidified incubator (37 °C, 5% CO_2).
   Note: *Cells may stick to bottom of the well. Mix gently using a serological pipette to ensure complete transfer.*

Day 7

9. Add 4 mL of pre-warmed 2X Complete ExCellerate Human NK Cell Expansion Media to each T25 flask. Total media should be 8 mL/flask. Place flasks vertically (vented flask cap facing up) in a humidified incubator (37 °C, 5% CO_2).

Day 9

10. Transfer the cell suspension (including Cloudz CD2/NKp46) from one T-25 flask into one T-75 flask. Add 8 mL of pre-warmed 2X Complete ExCellerate Human NK Expansion Media. Total media should be 16 mL/flask. Place flasks vertically (vented flask cap facing up) in a humidified incubator (37 °C, 5% CO_2).

Day 11

11. Carefully remove and discard 8 mL of media from each flask. Add 8 mL pre-warmed 2X Complete ExCellerate Human NK Cell Expansion Media to each flask. Total media should be 16 mL/flask. Place flasks vertically (vented flask cap facing up) in a humidified incubator (37 °C, 5% CO_2).

Day 14

12. Collect the cells from one T-75 flask into a 50 mL conical tube and centrifuge at 300 x g for 5 minutes.
13. Discard supernatant and add 10 mL of 1X Release Buffer. Gently pipette up and down 10 times to mix. Centrifuge at 300 x g for 5 minutes.
14. Discard supernatant and resuspend cells in 1X PBS.
15. To determine CD3 and CD56 expression on the cell surface, process, and stain in accordance with standard flow cytometry protocols.

ALTERNATIVE PROCEDURE: EX VIVO CULTURE OF HUMAN NK CELLS USING SERUM

REAGENT PREPARATION

Media A - Add Fetal Bovine Serum to Base Media at a final concentration of 10%. Immediately before use, add the following cytokines with the following recommended final concentrations: Recombinant Human IL-2 (27 ng/mL), Recombinant Human IL-12 (10 ng/mL), Recombinant Human IL-18 (10 ng/mL), and Recombinant Human IL-21 (10 ng/mL).

Media B - Add Fetal Bovine Serum to Base Media at a final concentration of 10%. Immediately before use, add the following cytokines with the following recommended final concentrations: Recombinant Human IL-2 (270 ng/mL), Recombinant Human IL-12 (20 ng/mL), Recombinant Human IL-18 (20 ng/mL), and Recombinant Human IL-21 (20 ng/mL).

1X Release Buffer - Immediately before use, dilute 6X Release Buffer 1:6 using Sterile 1X PBS to produce 1X Release Buffer. (*i.e.,* add 10 mL of 6X Release Buffer to 50 mL of Sterile 1X PBS to generate 1X Release Buffer).

The following protocol describes the expansion of human NK cells from PBMCs in T-25 flasks. This kit contains enough materials to activate and expand 50 x 10^6 NK cells from PBMCs, with greater than 150-fold expansion in 10 days. Expansion rates may vary by donor.
Note: *Cloudz Human NK Cell Expansion Kit is compatible for use with other combinations of cytokines and base media. If alternate cytokines, cytokine concentrations or media are desired, the expansion protocol may need to be optimized.*

ALTERNATIVE PROTOCOL

Day 0: Seeding of PBMCs in T-25 flasks with Cloudz CD2/NKp46:

1. Isolate and suspend PBMCs in Media A. Characterize the PBMCs to determine the starting population of $CD3^-/CD56^+$ NK cells.
2. In each T-25 flask, seed PBMCs containing 60,000 NK cells ($CD3^-/CD56^+$ cells). Add appropriate volume of Media A for a final T-25 flask volume of 8 mL.
3. Mix Cloudz CD2/NKp46 by vortexing the vial for 30 seconds.
4. Add 9 µL of Cloudz CD2/NKp46 directly into each flask. Gently pipet up and down 5 times to mix.
5. Place flasks vertically (vented flask cap facing up) in a humidified incubator (37 °C, 5% CO_2).

Day 3 - ½ Media Exchange with Media B:

1. Carefully remove and discard 4 mL of media from each flask.
   Note: *Avoid touching or disturbing cells at the bottom of the flask.*
2. Add 4 mL of pre-warmed Media B to each flask. Do not mix after media addition.
3. Place flasks vertically in a humidified incubator (37 °C, 5% CO_2).

Day 6 – Increase the Media in Flask with Media B:

1. Add 8 mL of pre-warmed Media B to each flask.
2. Place flasks vertically in a humidified incubator (37 °C, 5% CO_2).

Day 9 - ½ Media Exchange using Media B:

1. Remove and discard 8 mL of media from each flask.
   Note: *Avoid touching or disturbing cells at the bottom of the flask.*
2. Add 8 mL of Media B to each flask. Do not mix after media addition.
3. Place flasks vertically in a humidified incubator (37 °C, 5% CO_2).

Day 10 – Collect and Verify NK Cell Expansion via Flow Cytometry

1. Collect the cells from one T-25 flask into a 50 mL conical tube and centrifuge at 300 x g for 5 minutes.
2. Discard supernatant and add 10 mL of 1X Release Buffer. Gently pipette up and down 10 times to mix. Centrifuge at 300 x g for 5 minutes.
3. Discard supernatant and resuspend cells in 1X PBS.
4. To determine CD3 and CD56 expression on the cell surface, process and stain in accordance with standard flow cytometry protocols.
5. Analyze marker expression via flow cytometry as shown in the Data Examples.

DATA EXAMPLES

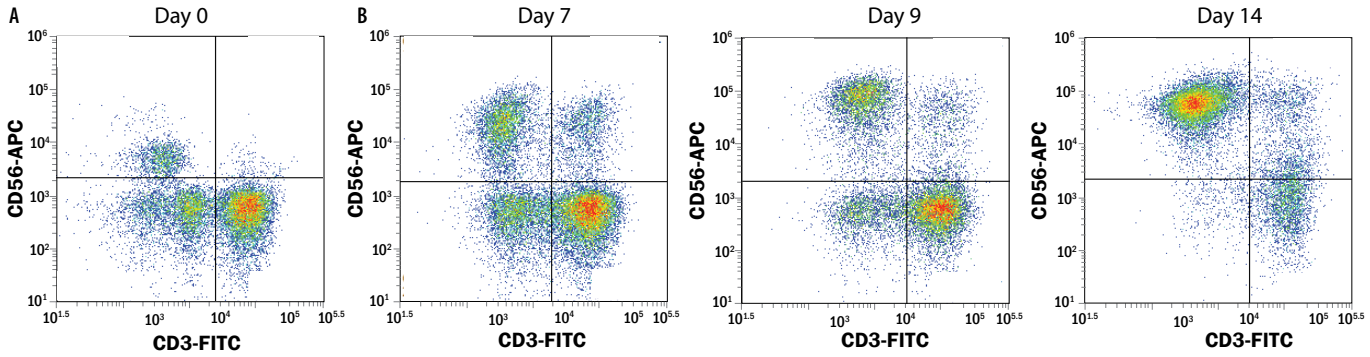


Figure 1: Flow Cytometry Analysis of Expanded Human NK Cells. Human PBMCs were expanded *in vitro* for 14 days using the serum-free protocol and reagents included in the Cloudz NK Cell Expansion Kit. At Day 7, 9, and 14 of expansion, NK cells were collected, stained with Human CD3 Fluorescent-conjugated antibody (R&D Systems, Catalog # FAB100F) and Human NCAM-1/CD56 APC-conjugate antibody, and analyzed by flow cytometry. Compared to unexpanded PBMCs on Day 0 (A), Days 7, 9 and 14 (B) show an increased number of $CD3^{-}CD56^{+}$ NK cells. Flow quadrants were set based on isotope-stained samples.

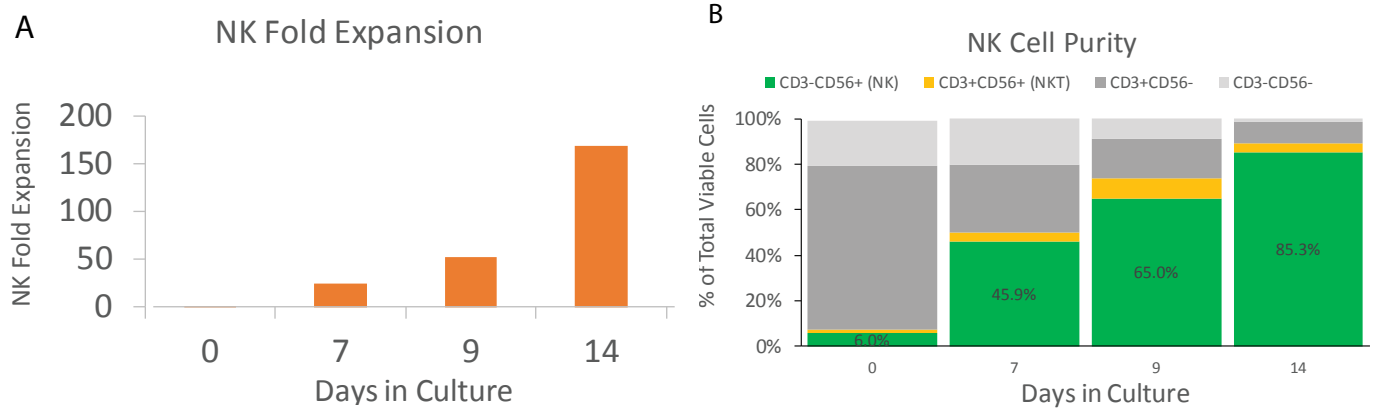


Figure 2: NK Cell Expansion and Purity Following Expansion using the Cloudz Human NK Cell Expansion Kit. Human PBMCs were expanded *in vitro* for 14 days using the serum-free protocol and reagents. Cells were evaluated at Days 0, 7, 9, and 14 for fold expansion (A) and population characterization (B). Following 14 days of expansion NK cells showed approximately 150-fold expansion (A) and a purity of 85.3% of $CD3^{-}CD56^{+}$ NK cells (B).

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.

NOTES

NOTES