ExCellerate™ Human NK Cell Expansion Media, Xeno-Free

Xeno-Free Media for Human Natural Killer Cell Culture

Catalog Number: CCM032

Volume: 500 mL

PRODUCT DESCRIPTION

Natural Killer (NK) cells play an important role in both the adaptive and innate immune responses that control infection, autoimmunity, and tumor immunosurveillance (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. Due to their intrinsic and non-specific anti-tumor activity, human NK cells have been employed as anti-cancer therapies (3-9). Furthermore, target-specific engineered NK cells that express Chimeric Antigen Receptors (CARs) enable greater precision in the treatment of malignancies (10-11). To facilitate discovery and preclinical research for NK cell immunoregulation and therapy, robust xeno-free and feeder-free platforms for *ex vivo* expansion and maintenance of NK cells are required.

Traditional methods for NK cell expansion employ serum-containing media or artificial antigen-presenting K562 feeder cells. Serum and feeder cells introduce uncontrolled variables into defined culture that can confound NK expansion and killing activity. ExCellerate Human NK Cell Expansion Media, Xeno-free, is specially formulated and optimized for the *ex vivo* feeder-free culture of human NK lymphocytes for research application. Unlike traditional serum-containing NK media, this xeno-free product provides a stable and reproducible cell culture environment for the expansion of NK cells under serum-free and feeder-free conditions. This product does not contain antibiotics or any non-human animal-derived components.

INTENDED USE

ExCellerate™ Human NK Cell Expansion Media, Xeno-Free, is a versatile media that can be used with a variety of cell activation methods and cytokines to culture and expand human NK cells *ex vivo*. The activation and cytokine/growth factor combination used with this media should be optimized by application or experimental protocol.

STABILITY & STORAGE

Upon receipt, this media should be stored at ≤ -20 °C. Upon thawing, store at 2-8 °C for up to 28 days.

PRECAUTIONS

The human origin-derived components used in this product have been derived from human plasma, which has been tested at the donor level and found to be negative for antibodies to HIV-1/2, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV). All other components are animal-free. The media should be handled as if potentially infectious. Safe laboratory procedures should be followed and protective clothing should be worn when handling this media. The acute and chronic effects of over-exposure to this media are unknown.

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

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.
- 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.

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THE FOLLOWING MATERIALS ARE REQUIRED FOR THE PROCEDURES BELOW

- 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)
- Cloudz™ Human NK Cell Expansion Kit (R&D Systems, Catalog # CLD004) or equivalent antibody-based stimulation method
- 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

REAGENT PREPARATION

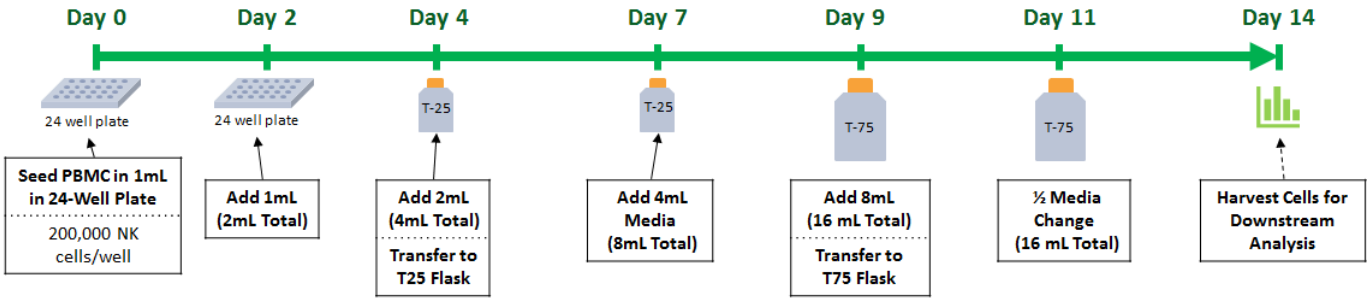
1X Complete ExCellerate Human NK Cell Expansion Media - Determine the amount of media needed for your experiment (*i.e.,* 1 mL of media is needed per well of a 24-well plate). Immediately before use, add the following cytokines with the following recommended final concentrations: Recombinant Human IL-2 (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 PROCEDURE FOR THE *EX VIVO* CULTURE OF HUMAN NK CELLS USING CLOUDZ NK CELL EXPANSION KIT

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% CO2).

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 PROTOCOL USING PLATE-BOUND ANTIBODY

Day 0

1. Pre-warm the required amount of 1X Complete ExCellerate™ Human NK Cell Expansion Media to room temperature.
2. Pre-coat a 24-well tissue culture plate with 10 µg/mL of Human NKp46/NCR1 Antibody (R&D Systems®, Catalog # MAB1850) in 1X PBS (250 µL per well to ensure well is completely covered).
3. Incubate plate for 1-3 hours at 37 °C.
4. Wash plate with 1 mL/well of sterile 1X PBS.
5. Isolate PBMCs or $CD3^+$-depleted PBMCs using desired protocol. Determine the concentration of (NK cells/mL) in the starting population using flow cytometry.
6. Dilute cell suspension to approximately 0.2 x 10^6 NK cells/mL in 1X Complete ExCellerate Human NK Cell Expansion Media. Add 1 mL of cells to each well.
7. Incubate at 37 °C and 5% CO_2 in a humidified incubator.

Day 2

8. Feed cells with 1 mL 2X Complete ExCellerate Human NK Cell Expansion Media.
   Note: *In general, NK cells will need a media change media every 2-3 days.*

Day 5

9. Pre-coat T25 flasks with 10 µg/mL of Human NKp46/NCR1 Antibody in 1X PBS (1 mL/flask to ensure flask bottom is completely coated).
10. Incubate flask for 1-3 hours at 37 °C.
11. Wash flask with > 2 mL per flask of sterile 1X PBS.
12. Move cell suspension to a pre-coated T25 flask using a serological pipette.
13. Feed each flask with 2 mL of 2X Complete ExCellerate Human NK Cell Expansion Media.
14. Place flasks in a humidified incubator (37 °C, 5% CO_2).

Day 8

15. Feed each flask with 4 mL of 2X Complete ExCellerate Human NK Cell Expansion Media.
16. Place flasks in a humidified incubator (37 °C, 5% CO_2).

Day 11

17. Pre-coat T75 flask with 10 µg/mL Human NKp46/NCR1 Antibody in 1X PBS (2 mL/flask to ensure the bottom is completely coated).
18. Incubate flask for 1-3 hours at 37 °C.
19. Wash flask with > 4 mL per well of sterile 1X PBS.
20. Move cell suspension to a pre-coated T75 flask using a serological pipette.
21. Feed each flask with 8 mL of 2X Complete ExCellerate Human NK Cell Expansion Media.
22. Place flasks in a humidified incubator (37 °C, 5% CO_2).

    Note: *Some donors exhibit above average growth rates, which may require splitting the NK cells or additional feeding steps. If the culture media begins to turn yellow, this indicates cell overgrowth and we recommend splitting the NK cells 1:2 or 1:4 to obtain an optimal culture density of 1 x 10^6 cells/mL.*

Day 14

23. Harvest the cells on Day 14 for desired downstream applications.

DATA EXAMPLES

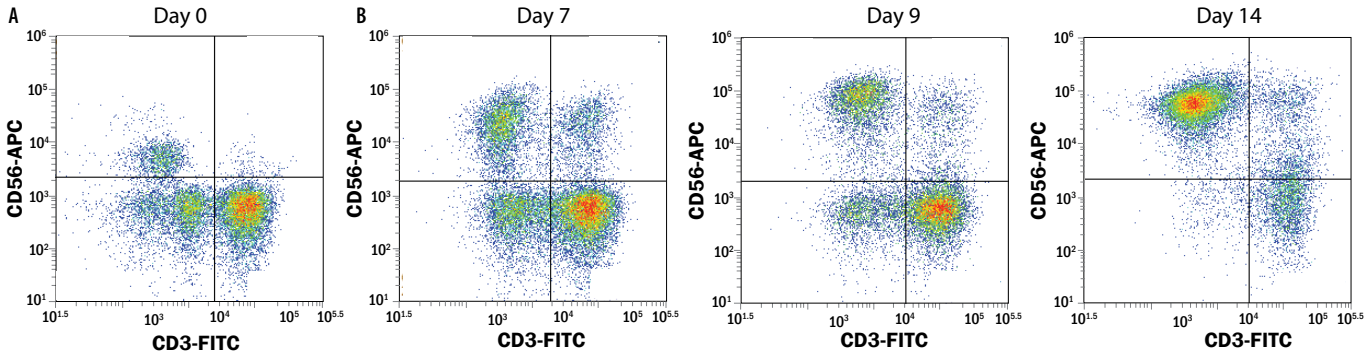


Figure 1: Flow Cytometry Analysis of Expanded Human NK Cells. Human PBMCs were expanded *in vitro* for 14 days using the protocol and reagents included in the Cloudz NK Cell Expansion Kit. At Day 7, 9, and 14 of expansion, NK cells were collected, stained with Human CD3 Fluorescent-conjugated antibody (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.

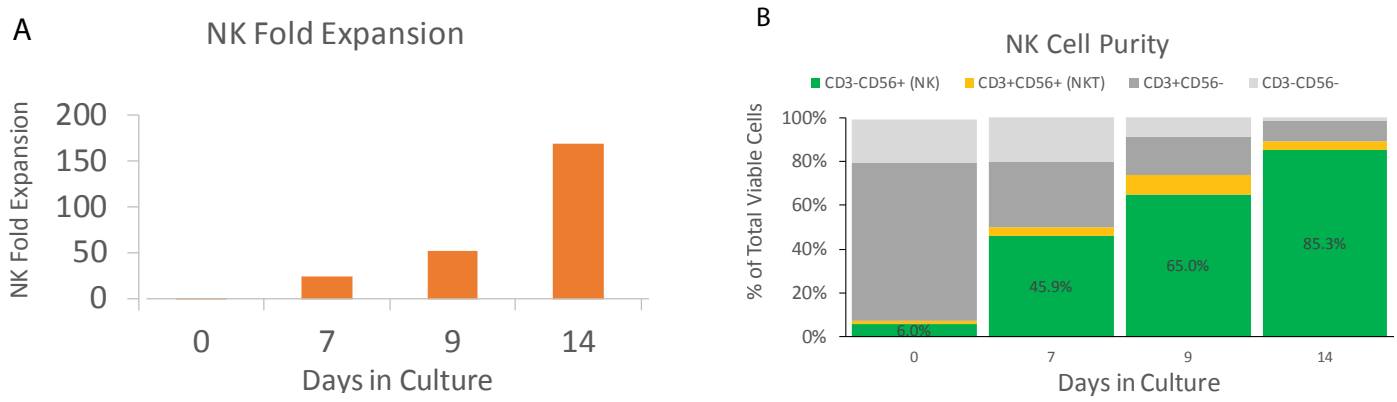


Figure 2: NK Cell Expansion and Purity Following Expansion using the Cloudz Human NK Cell Expansion Kit. Human PBMCs were expanded *in vitro* for 14 days using the Cloudz NK Expansion Protocol and reagents. Cells were evaluated at Days 0, 7, 9, and 14 for fold expansion (A) and population characterization (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).

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