**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 IFN-γ, TNF-β, TNF-α, GM-CSF, 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).

ExCellerate Human NK Cell Expansion Media, Animal Component-Free, is specially formulated and optimized for the ex vivo expansion culture of human NK lymphocytes for research application.

**INTENDED USE**

ExCellerate Human NK Cell Expansion Media, Animal Component-Free, can be used with a variety of cell activation methods and cytokines to culture and expand human NK cells ex vivo. It can be used in the absence of serum or feeder cells in certain culture conditions. The culture conditions 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**

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.
- 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 FOLLOWING MATERIALS ARE REQUIRED**

- Human NKp46/NCR1 Antibody
  (R&D Systems®, Catalog # MAB1850)
- Recombinant Human IL-2
  (R&D Systems, Catalog # BT-002-AFL)
- 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)
- Sterile Phosphate Buffered Saline (PBS)
- Pipettes and pipette tips
- 15 mL and 50 mL Polypropylene Centrifuge Tubes
- 96-well tissue culture plate
- 24-well tissue culture plate
- 25 cm2 tissue culture flask (T25)
- 75 cm2 tissue culture flask (T75)
- 37 °C, 5% CO2 incubator
- Inverted microscope
- Flow cytometer
- Cell counting materials
- Centrifuge
**REAGENT PREPARATION**

**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).

**RECOMMENDED PROTOCOL (T75 Flasks)**

*Figure 1. The protocol for the expansion of human NK cells using ExCellerate Human NK Cell Expansion Media, Animal Component-Free:*

**Day 0**
1. Pre-warm the required amount of Complete ExCellerate Human NK Cell Expansion Media to room temperature.
2. Pre-coat a 96-well tissue culture plate with 10 μg/mL of Human NKp46/NCR1 Antibody in 1X PBS (50 μL per well to ensure well is completely covered).
3. Incubate plate for 2 hours at 37 °C.
4. Wash plate with 250 μL /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 2 x 10⁵ NK cells/mL in Complete ExCellerate Human NK Cell Expansion Media. Add 250 μL of cells to each well.
7. Place plate in a humidified incubator.

**Day 3**
8. Pre-coat a 24-well tissue culture plate with 10 μg/mL of Human NKp46/NCR1 Antibody in 1X PBS (250 μL per well to ensure well is completely covered).
9. Incubate plate for 2 hours at 37 °C.
10. Wash plate with 1 mL/well of sterile 1X PBS.
11. Move cell suspension to the coated 24-well plate. Wash each well of the 96-well plate with 250 μL Complete ExCellerate Human NK Cell Expansion Media to transfer the residual cells to the coated 24-well plate.
12. Feed cells with 1.5 mL Complete ExCellerate Human NK Cell Expansion Media.
13. Place plate in a humidified incubator.
RECOMMENDED PROTOCOL (T75 Flasks) CONTINUED

Day 7
14. Pre-coat T25 flask with 10 μg/mL of Human NKp46/NCR1 Antibody in 1X PBS (2 mL/flask to ensure flask bottom is completely coated).
15. Incubate flask for 2 hours at 37 °C.
16. Wash flask with 5 mL of sterile 1X PBS.
17. Move cell suspension to the pre-coated T25 flask. Wash each well of the 24-well plate with 1 mL Complete ExCellerate™ Human NK Cell Expansion Media to transfer the residual cells to the coated T25 flask.
18. Feed the flask with 5 mL of Complete ExCellerate Human NK Cell Expansion Media.
19. Place flask in a humidified incubator.

Day 9
20. Pre-coat T75 flask with 10 μg/mL of Human NKp46/NCR1 Antibody in 1X PBS (3 mL/flask to ensure flask bottom is completely coated).
21. Incubate flask for 2 hours at 37 °C.
22. Wash flask with 5 mL of sterile 1X PBS.
23. Move cell suspension to the pre-coated T75 flask. Wash T25 flask with 2 mL Complete ExCellerate Human NK Cell Expansion Media to transfer the residual cells to the coated T75 flask.
24. Feed the flask with 6 mL of Complete ExCellerate Human NK Cell Expansion Media.
25. Place flask in a humidified incubator.

Day 10
26. Split cells from T75 flask into two T75 flasks.
27. Feed each flask with 8 mL of Complete ExCellerate Human NK Cell Expansion Media.
28. Place flasks in a humidified incubator.

Day 11
29. Split cells in each T75 flask into four T75 flasks.
30. Feed each flask with 12 mL of Complete ExCellerate Human NK Cell Expansion Media.
31. Place flasks in a humidified incubator.

Day 14
32. Harvest cells for the downstream analysis and applications.

REFERENCES