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# Human NK Cell Expansion Super Kit

**Product Number: CEK004**

## Shipping and Storage

Store at -20°C . Unused factors should be stored in a -20°C freezer and thawed in a 4°C refrigerator when needed.

## Component

| Component | CEK004 |
|---|---|
| NK Cell Expander 1 | 1 |
| NK Cell Expander 2 | 1 |
| NK Cell Expander 3 | 1 |
| NK Cell Expander 4 | 1 |

## Description

This kit is designed for the highly efficient activation and large-scale expansion of human natural killer (NK) cells in vitro. By integrating critical signaling pathway factors essential for NK cell activation with key cytokines crucial for maintaining cell viability and function, it enables the rapid production of high-purity, highly active NK cells from both frozen and fresh peripheral blood mononuclear cells (PBMCs) or cord blood mononuclear cells (CBMCs). It serves as a powerful tool for tumor immunotherapy research.

## Application

1. NK cell research for cancer immunotherapy
2. CAR-NK cell therapy development
3. Functional and biological studies of NK cells
4. Large-scale preparation of NK cells for in vitro cytotoxicity assays

## Features

1. High Expansion Efficiency: Proprietary formulation of cytokines and activation factors supports up to approximately 1000-fold expansion of NK cells within 18 days.
2. High Target Cell Purity: The optimized system effectively promotes the specific proliferation of NK cells, yielding a final population of high purity.
3. Excellent Media Compatibility: Compatible with a wide range of common basal media, offering flexibility for your experimental protocol.
4. Defined, Serum-Free Formulation: Uses a pure protein factor approach, ensuring consistency, reliability, and enhanced safety by eliminating variability associated with serum.

## Protocol

1. **Coating:**

   Reconstitute NK Cell Expander 1 using 1mL of DPBS. Add 14mL of DPBS and the reconstituted NK Cell Expander 1 to a T175 flask. Mix thoroughly and incubate overnight at 4°C for 16-24 hours. After incubation, aspirate the supernatant and wash once with 10mL of DPBS. Take care not to disrupt the coated surface at the bottom of the flask. Discard the wash solution and set the flask aside for later use.

2. **Culture:**

   **2.1. Day 0:**

   PBMC Seeding: Add 45mL of serum-free medium for immune cells, 1 vial of reconstituted NK Cell Expander 2, 10%

autologous plasma (5mL), and mononuclear cells to the T175 flask. Mix gently and culture in a 37°C, 5% $CO_2$ incubator. The total volume is 50mL. (This volume is suitable for culturing mononuclear cells isolated from 50-100mL of peripheral blood. The recommended initial seeding density for PBMCs isolated from peripheral blood is not less than $2x10^6$/mL. The recommended initial seeding density for Cord Blood Mononuclear Cells (CBMCs) is not less than $3x10^6$/mL).

**2.2. Day 1:**

Reconstitute one vial of NK Cell Expander 3 with 1mL of immune cell medium. Add the entire contents of the reconstituted NK Cell Expander 3 to the T175 flask. After adding, gently mix and continue culturing in the 37°C, 5% $CO_2$ incubator.

**2.3. Day 3:**

Reconstitute one vial of NK Cell Expander 3 with 1mL of immune cell medium. Add 44mL of immune cell medium, the reconstituted NK Cell Expander 3, and 10% autologous plasma (5mL) to the T175 flask. Perform this addition gently to avoid dislodging the cells. The total volume in the T175 flask is now 100mL. (Note: Medium replenishment must be done gently to avoid cell dislodgement. It is advisable not to frequently observe the cells during the first 3 days of culture; let them settle undisturbed.).

**2.4. Day 5:**

Preparation of Expansion Medium: Reconstitute one vial of NK Cell Expander 4 with 1mL of immune cell medium. Add 500µL of the reconstituted NK Cell Expander 4 to 1 L of immune cell medium and mix well. Store the remaining 500µL of NK Cell Expander 4 at 4°C (can be stored for approximately 20 days). Use aseptically. The prepared expansion medium can be stored at 4°C for approximately 20 days. Use the first bottle of expansion medium before opening the next.

Add 90mL of expansion medium and 10% autologous plasma (10mL) to the T175 flask. The total volume in the flask is now 200mL.

**2.5. Day 7:**

Transfer: Gently resuspend the cells in the T175 flask. Perform a cell count and transfer the entire cell suspension to a cell culture bag. Rinse the T175 flask with 200mL of expansion medium and transfer the rinse medium to the cell culture bag to minimize NK cell loss. Then, add 180mL of expansion medium and 5% autologous plasma (20mL) to the cell culture bag. If less than 20mL of autologous plasma remains, add the entire remaining volume. The total volume in the culture bag is now approximately 600mL. (If the cell count is below $1.5x10^6$/mL, after resuspending and transferring the cells to the bag, rinse the T175 flask with 190mL of expansion medium, transfer the rinse medium to the bag, and then add 5% autologous plasma (10mL) to the bag.)

**2.6. Day 9-Day 13:**

Depending on cell growth status (determined by cell counting), it is recommended to remove 500 mL of expansion medium from the cell culture bag every two days.

**2.7. Day 14 - Day 21:**

Monitor cell density as required and harvest the cells.