EIV22024E

ExCell Bio

OptiVitro® NK Cell Expansion Kit P01

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number	NE000-N022
Catalog Number	NE000-N021
Catalog Number	NE000-N021S

PRODUCT DESCRIPTION

OptiVitro® NK Cell Expansion Kit P01 is a comprehensive, serum-free, and xeno-free kit designed for the expansion and culture of NK cells. This kit encompasses everything needed for the robust expansion of NK cells, including:

- **OptiVitro® NK Cell Serum-free Basal Medium P01:** Providing a foundational medium for NK cell growth.
- **OptiVitro® Immune Cell Serum-free Medium Supplement UE01:** Enhancing the basal medium with essential nutrients.
- **OptiVitro® Cytokine I, Cytokine II, and Cytokine III:** Crucial components for supporting NK cell activation and proliferation.

This kit is meticulously formulated to support the selective expansion of NK cells derived from human peripheral blood mononuclear cells (PBMC) and umbilical cord blood mononuclear cells. It is also compatible with NK cells differentiated from induced pluripotent stem cells (iPSC) and established NK cell lines, making it a versatile solution for various research and manufacturing applications.

SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

Product Name	Cat.#	Specification	Storage	Transportation	Shelf Life
OptiVitro® NK Cell Expansion Kit P01	**NE000-N022**	**1000 mL kit**	**2-8°C Protect From Light**	-	-
OptiVitro® NK Cell Serum-free Basal Medium P01	BA0092	1000 mL	2-8°C Protect From Light	< 25°C Protect From Light	12 months
OptiVitro® Immune Cell Serum-free Medium Supplement UE01	BA0332	8 mL	2-8°C Protect From Light	< 25°C Protect From Light	18 months
OptiVitro® Cytokine I	BA0112	45 uL	-20°C Protect From Light	< 0°C Protect	12 months

				From Light	
OptiVitro® Cytokine II	BA0122	150 uL	-20℃ Protect From Light	< 0℃ Protect From Light	12 months
OptiVitro® Cytokine III	BA0132	310 uL	-20℃ Protect From Light	< 0℃ Protect From Light	12 months
OptiVitro® NK Cell Expansion Kit P01	**NE000-N021**	**500 mL kit**	**2-8℃ Protect From Light**	-	-
OptiVitro® NK Cell Serum-free Basal Medium P01	BA0091	500 mL	2-8℃ Protect From Light	< 25℃ Protect From Light	12 months
OptiVitro® Immune Cell Serum-free Medium Supplement UE01	BA0331	4 mL	2-8℃ Protect From Light	< 25℃ Protect From Light	18 months
OptiVitro® Cytokine I	BA0111	22.5 uL	-20℃ Protect From Light	< 0℃ Protect From Light	12 months
OptiVitro® Cytokine II	BA0121	75 uL	-20℃ Protect From Light	< 0℃ Protect From Light	12 months
OptiVitro® Cytokine III	BA0131	155 uL	-20℃ Protect From Light	< 0℃ Protect From Light	12 months
OptiVitro® NK Cell Expansion Kit P01	**TE000-N021S**	**100 mL kit**	**2-8℃ Protect From Light**	-	-
OptiVitro® NK Cell Serum-free Basal Medium P01	BA0141S	100 mL	2-8℃ Protect From Light	< 25℃ Protect From Light	12 months
OptiVitro® Immune Cell Serum-free Medium Supplement UE01	BA0331S	0.8 mL	2-8℃ Protect From Light	< 25℃ Protect From Light	18 months
OptiVitro® Cytokine I	BA0111S	9 uL	-20℃ Protect From Light	< 0℃ Protect From Light	12 months
OptiVitro® Cytokine II	BA0121S	30 uL	-20℃ Protect From Light	< 0℃ Protect From Light	12 months

OptiVitro® Cytokine III	BA0131S	31 uL	-20℃ Protect From Light	< 0℃ Protect From Light	12 months

PERFORMANCE, APPLICATION AND HANDLING RECOMMENDATIONS

1. Store cell culture medium in a dark environment, ideally in colored packaging, to protect it from light exposure.

2. Avoid prolonged exposure to fluorescent or other types of lighting during transport to prevent discoloration.

3. Implement thorough cleaning and sterilization methods for transport to sterile areas; avoid UV sterilization.

4. Switch off UV lamps when transferring through UV-sterilized windows.

EXPERIMENTAL MATERIALS AND REAGENTS

1. Peripheral blood mononuclear cells (PBMC) or umbilical cord blood mononuclear cells.

2. Heat-inactivated autologous plasma (commercial serum substitutes or human AB serum can also be used).

3. Culture plates/culture bottles/culture bags.

4. Lymphocyte separation fluid, DPBS solution or saline, centrifuge tubes, pipettes, pipette guns, and pipette tips.

5. CO_2 incubator, centrifuge, cell counter, inverted microscope, water bath, etc.

Prepare media

1. Equilibrate OptiVitro® NK Cell Serum-free Basal Medium P01 and OptiVitro® Immune Cell Serum-free Medium Supplement UE01 at room temperature for 1-4 hours. In a biosafety cabinet, add 8 mL/4 mL of supplement to every 1 L/500 mL of basal medium, mix by inverting 3-5 times to obtain the complete medium.

2. Add 1 vial of 310 μL/155 μL Cytokine III to every 1000 mL/500 mL complete OptiVitro® NK Cell Serum-free Basal Medium P01 to prepare the complete medium for NK cell expansion (referred to as NK complete medium). The shelf life after preparation is 3 weeks. Cytokine III can be aliquoted to extend usage time, with no more than 3 freeze-thaw cycles.

【Note】

Allow cytokines to thaw at room temperature for about 10 minutes before use, and avoid prolonged exposure to air once the container is opened.

Activation and Expansion of NK Cells from PBMC:

1. Day0

Pretreatment of T75 culture flask: Thaw Cytokine I at room temperature, dilute 45 μL Cytokine I in 15 mL DPBS, and coat a T75 culture flask at 4°C overnight or at 37°C for at least 2 hours for emergency coating.

PBMC inoculation: Remove coating solution from the activated T75 flask, add NK complete medium, 150 μL of Cytokine II, 10% autologous plasma (1.5 mL), and seed cells with a total volume of 15 mL. Shake and place in a 37°C, 5% CO_2 incubator.

【Note】

1. Remove the coated culture flask and discard the coating solution 10 minutes before cell inoculation.

2. The recommended starting cell density for PBMC seeding is 2-2.5×10^6 cells/mL. For cord blood with a low initial NK ratio, increase to 3×10^6 cells/mL.

3. Use an electric pipette to seed cells, avoiding contact with the coating and spreading evenly.

2. Day3

Slowly add 13.5 mL of NK complete medium and 10% heat-inactivated autologous plasma (1.5 mL) along the side wall of the culture flask.

3. Day5

Sample, count, and add fresh NK complete medium (with 5% heat-inactivated autologous plasma), adjusting cell density to 1.0×10^6 cells/mL. Transfer the culture medium and cells from the T75 to a T175 flask.

4. Day 7 and beyond

Sample and count every 1-2 days for fluid replenishment, adjusting cell density to $0.5\text{-}1.0\times10^6$ cells/mL. Expand in a larger bottle or transfer to a cell culture bag as needed. From Day 7, reduce heat-inactivated autologous plasma in supplemented fresh NK complete medium to 1%.

5. Harvest cells

Harvest cells on days 14-18.

6. OptiVitro® NK Cell Expansion Basic Kit P01 (NE000-N03#) contains OptiVitro® NK Cell Serum-free Basal Medium P01, supplement and cytokine III, which can be used in conjunction with this kit to support larger batches of NK cell expansion needs after NK cell activation.

OTHERS

1. Adjust cytokine amounts for smaller system testing as per the provided table:

Specification	Cytokine I	Coating volume	Cytokine II	PBMC seeding density	Inoculation volume
T75	45 μL	15 mL	150 μL	$1.5\text{-}2.5\times10^6$ cells/mL	15 mL
T25	15 μL	5 mL	50 μL	$1.5\text{-}2.5\times10^6$ cells/mL	5 mL
6-well plate (per well)	6 μL	2 mL	20 μL	$1.5\text{-}2.5\times10^6$ cells/mL	2 mL
12-well plate (per well)	3 μL	1 mL	10 μL	$1.5\text{-}2.5\times10^6$ cells/mL	1 mL

2. The cell seeding density on day 0 can be $1.5\text{-}2.5\times10^6$ cells/mL. For frozen PBMC, thaw one day in advance and rest overnight in the incubator.

3. Inoculation density lower than 1.0×10^6 cells/mL may lead to culture failure.

4. If NK cells are isolated from PBMCs before culturing, the seeding density can be $1.0\text{-}2.0\times10^6$ cells/mL.

DISCLAIMER

1. Use the product according to the manual instructions. Deviations from these instructions are at the user's risk, and our company will not be responsible for any resulting product performance deviations.

2. This product is for scientific research and commercial production only and is not intended for clinical diagnosis or treatment. Users assume all risks for unauthorized use, and our company shall not be responsible for any consequences.