EIV22024E

ExCell Bio

OptiVitro® NK Cell Expansion Basic Kit NE01 (phenol red-free)

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number	NE000-N062
Catalog Number	NE000-N061
Catalog Number	NE000-N061S

PRODUCT DESCRIPTION

OptiVitro® NK Cell Expansion Basic Kit NE01 (Phenol Red-Free) is a serum-free, and xeno-free culture system specifically designed for the robust expansion of activated natural killer (NK) cells. This comprehensive kit includes:

- **OptiVitro® NK Cell Serum-free Basal Medium NE01 (Phenol Red-Free):** 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 III:** A crucial component for supporting NK cell 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.

Key Features:

- **Activation Compatibility:** The kit is designed to work seamlessly with OptiVitro® Cytokine I and Cytokine II for the activation of NK cells, ensuring a streamlined process from activation to expansion.
- **Alternative Activation Methods:** While formulated to work with our cytokines, the kit is also adaptable for use with alternative methods of NK cell activation, providing flexibility for various experimental designs.
- **Expansion of Activated NK Cells:** Primarily supports the expansion of already activated NK cells, making it an ideal choice for applications where NK cells have been pre-activated through different stimulation methods.
- **iPSC-derived NK Cells:** Effective for the expansion of NK cells derived from iPSC differentiation, facilitating research into immune cell therapy and regenerative medicine.
- **NK Cell Lines:** Suitable for the expansion of established NK cell lines, like NK92, supporting consistent and reliable results in immunological research and bioproduction.

| SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

Product Name	Cat.#	Specification	Storage	Transportation	Shelf Life
OptiVitro® NK Cell Expansion Basic Kit NE01 (phenol red-free)	**NE000-N062**	**1000 mL kit**	**2-8 ℃ Protect from light**	-	-
OptiVitro® NK Cell Serum-free Basal Medium NE01 (phenol red-free)	BA0142	1000 mL	2-8 ℃ Protect from light	< 25 ℃ Protect from light	12 months
OptiVitro® Immune Cell Serum-free Medium Supplement UE01	BA0332	8 mL	2-8 ℃ Protect from light	< 25 ℃ Protect from light	18 months
OptiVitro® Cytokine III	BA0132	310 μL	-20 ℃	< 0 ℃ Protect from light	12 months
OptiVitro® NK Cell Expansion Basic Kit NE01 (phenol red-free)	**NE000-N061**	**500 mL kit**	**2-8 ℃ Protect from light**	-	-
OptiVitro® NK Cell Serum-free Basal Medium NE01 (phenol red-free)	BA0141	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 III	BA0131	155 uL	-20 ℃	< 0 ℃ Protect from light	12 months
OptiVitro® NK Cell Expansion Basic Kit NE01 (phenol red-free)	**NE000-N061S**	**100 mL kit**	**2-8 ℃ Protect from light**	-	-
OptiVitro® NK Cell Serum-free Basal Medium NE01 (phenol red-free)	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 III	BA0131S	31 uL	-20 ℃	< 0 ℃ Protect from light	12 months

PERFORMANCE, APPLICATION AND HANDLING RECOMMENDATIONS

1. Store cell culture medium in a dark environment, preferably in colored packaging, to protect from light exposure.
2. Avoid prolonged exposure to 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 NE01 and OptiVitro® Immune Cell Serum-Free Medium Supplement UE01 to room temperature for 1-4 hours.

2. 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.

3. Add one vial of 310 µL/155 µL Cytokine III to every 1000 mL/500 mL of complete medium to prepare the NK cell expansion medium. The medium is stable for 3 weeks after preparation.

Activation and expansion of NK cells from PBMC

1. Day0

Flask Preparation: If necessary for your specific NK cell activation protocol, coat the T75 culture flask as required.

Cell Seeding: In the T75 flask, combine NK culture medium, 10% heat-inactivated autologous plasma (1.5 mL), and any additional NK activation reagents as needed. Seed the PBMCs into the flask to achieve a total volume of 15 mL. Gently shake the flask to distribute the cells evenly and place it in a 37°C, 5% CO_2 incubator.

【Note】

Recommended starting cell density for PBMC seeding is 2-2.5×10^6 cells/mL. For cord blood with low initial NK ratio, increase to 3×10^6 cells/mL. Use an electric pipette to seed cells, avoiding contact with the coating and spreading evenly.

2. Day3

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

3. Day5

Sample and count, adjust cell density to 1.0×10^6 cells/mL, and transfer to a T175 culture flask.

4. Day 7 and beyond

Sample and count every 1-2 days, adjust cell density to 0.5-1.0×10^6 cells/mL, and expand or transfer to a cell culture bag as needed. Reduce heat-inactivated autologous plasma to 1% from Day 7.

5. Harvest cells

Harvest cells on days 14-18.

OTHERS

1. If NK cells are isolated from PBMCs before culturing, the seeding density can be 1.0-2.0×10^6 cells/mL.

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

DISCLAIMER

1. Use the product according to the manual instructions. Deviations from these instructions are at the user's risk.

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.