EIV32025E

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
	NE000-N061
	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 (PBMCs) and umbilical cord blood mononuclear cells (CBMCs). It is also compatible with NK cells differentiated from induced pluripotent stem cells (iPSCs) 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 OptiVitro® Cytokine II (from OptiVitro® NK Cell Expansion Kit NE01 (phenol red-free) (NE000-N052)) 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**	-	-	-
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℃ Protect From Light.	< 0℃ Protect From Light.	12 months
OptiVitro® NK Cell Expansion Basic Kit NE01 (phenol red-free)	**NE000-N061**	**500 mL kit**	-	-	-
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 μL	-20℃ Protect From Light.	< 0℃ Protect From Light.	12 months
OptiVitro® NK Cell Expansion Basic Kit NE01 (phenol red-free)	**NE000-N061S**	**100 mL kit**	-	-	-
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 μL	-20℃ Protect From Light.	< 0℃ Protect From Light.	12 months

HANDLING RECOMMENDATIONS

1. Please make sure to store the cell culture medium in a light-protected environment, avoid fluorescent lamps or other lamplight exposure, and better to use colored packaging bags in the refrigerator or warehouse.
2. During the transportation of the product, it should be kept away from light. This is to prevent the product from being affected by the irradiation of fluorescent lamps or other light sources, which may lead to discoloration.
3. During the transportation of the product to the clean area, it is essential to carry out a cleaning process. The cleaning method may involve disinfectant wiping, and not utilize UV irradiation.

   Note: *When passing through transfer windows equipped with UV lamp, remember to proactively turn off the UV lamp inside the transfer window.*
4. The medium can be used with heat-inactivated autologous plasma, commercial human platelet lysate products, or human AB serum, but not with ICSR products.

Prepare media

1. Equilibrate OptiVitro® NK Cell Serum-free Basal Medium NE01 (phenol red-free) 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 1000 mL/500 mL of basal medium, mix by inverting 3-5 times to obtain the complete OptiVitro® NK Cell Serum-free Basal Medium NE01 (phenol red-free).
2. Add 1 vial of 310 µL/155 µL OptiVitro® Cytokine III to every 1000 mL/500 mL complete OptiVitro® NK Cell Serum-free Basal Medium NE01 (phenol red-free) to prepare the complete medium for NK cell expansion (referred to as NK complete medium below). The shelf life after preparation is two weeks. OptiVitro® Cytokine III can be aliquoted to extend usage time, with no more than 3 freeze-thaw cycles.

Note:

1) Allow the basal medium and supplement to equilibrate to room temperature for 1-4 hours before mixing.
2) Once mixed, the medium can be stored at 2-8°C, protect from light, and should be used within two weeks.
3) A small amount of precipitation in the supplement when stored at 2-8°C is normal and does not affect the effects. The precipitation will dissolve after 1-4 hours at room temperature.
4) Allow cytokines to thaw at room temperature for about 10 minutes before use, briefly centrifuge the vial before use.

Activation and Expansion of NK Cells from PBMCs:

Day0

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

Cell seeding: In the T75 flask, combine NK complete 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:

1) The recommended starting cell density for PBMCs seeding is 2.0-2.5×10^6 cells/mL. For CBMCs with a low initial NK ratio, increase the seeding density to 3.0×10^6 cells/mL. Seeding density lower than 1.0×10^6 cells/mL may lead to culture failure.
2) Use an electric pipette to seed cells, avoiding contact with the coating and spreading evenly.
3) If NK cells are isolated from PBMCs before culturing, the seeding density can be 1.0-2.0×10^6 cells/mL.

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.

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.

Day 7 and beyond

Sample and count every 1-2 days for fluid replenishment, adjusting cell density to 0.5-1.0×10^6 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%.

Harvest cells

Harvest cells on days 14-18.

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.