EIV32025E

ExCell Bio

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

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number	NE000-N052
	NE000-N051
	NE000-N051S

ExCell

PRODUCT DESCRIPTION

OptiVitro® NK Cell Expansion Kit NE01 (phenol red-free) 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 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 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 (PBMCs) and umbilical cord blood mononuclear cells (CBMCs). 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 NE01 (phenol red-free)	**NE000-N052**	**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 I	BA0112	45 μL	-20℃ Protect From Light.	< 0℃ Protect From Light.	12 months
OptiVitro® Cytokine II	BA0122	150 μL	-20℃ Protect From Light.	< 0℃ Protect From Light.	12 months
OptiVitro® Cytokine III	BA0132	310 μL	-20℃ Protect From Light.	< 0℃ Protect From Light.	12 months
OptiVitro® NK Cell Expansion Kit NE01 (phenol red-free)	**NE000-N051**	**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 I	BA0111	22.5 μL	-20℃ Protect From Light.	< 0℃ Protect From Light.	12 months
OptiVitro® Cytokine II	BA0121	75 μL	-20℃ Protect From Light.	< 0℃ Protect From Light.	12 months
OptiVitro® Cytokine III	BA0131	155 μL	-20℃ Protect From Light.	< 0℃ Protect From Light.	12 months
OptiVitro® NK Cell Expansion Kit NE01 (phenol red-free)	**NE000-N051S**	**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 I	BA0111S	9 μL	-20℃ Protect From Light.	< 0℃ Protect From Light.	12 months
OptiVitro® Cytokine II	BA0121S	30 μL	-20℃ Protect From Light.	< 0℃ Protect From Light.	12 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

Pretreatment of T75 culture flask: Thaw OptiVitro® Cytokine I at room temperature, dilute 45 µL OptiVitro® 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.

PBMCs seeding: Remove coating solution from the activated T75 flask, add NK complete medium, 150 µL of OptiVitro® 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 seeding cells.
2) 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.
3) Use an electric pipette to seed cells, avoiding contact with the coating and spreading evenly.

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.

Note: *OptiVitro® NK Cell Expansion Basic Kit NE01 (phenol red-free) (NE000-N062) contains OptiVitro® NK Cell Serum-free Basal Medium NE01 (phenol red-free), OptiVitro® Immune Cell Serum-free Medium Supplement UE01 and OptiVitro® 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

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

Specification	Cytokine I	Coating volume	Cytokine II	PBMCs seeding density	Seeding volume
T75	45 µL	15 mL	150 µL	2.0-2.5×10^6 cells/mL	15 mL
T25	15 µL	5 mL	50 µL	2.0-2.5×10^6 cells/mL	5 mL
6-well plate (per well)	6 µL	2 mL	20 µL	2.0-2.5×10^6 cells/mL	2 mL
12-well plate (per well)	3µL	1 mL	10 µL	2.0-2.5×10^6 cells/mL	1 mL

Note:

1) *The cell seeding density on day 0 can be 2.0-2.5×10^6 cells/mL, for frozen PBMCs, the seeding density can be increased to 2.0-3.0×10^6 cells/mL.*
2) *Inoculation density lower than 1.0×10^6 cells/mL may lead to culture failure.*
3) *If NK cells are isolated from PBMCs before culturing, the seeding density can be 1.0-2.0×10^6 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.