EIV12023E

ExCell Bio

OptiVitro® NK Cell Expansion Serum-free Basic Kit P01

For Research and Manufacturing Use
Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number NE000-N032
NE000-N031
NE000-N031S

Product description

OptiVitro® NK Cell Expansion Serum-free Basic Kit P01 has been specifically designed for the in vitro expansion of human Natural Killer (NK) cells derived from either peripheral blood mononuclear cells (PBMCs) or cord blood mononuclear cells (CB-MNCs). The kit is composed of three main components: OptiVitro® NK Cell Basal SF Medium P01, OptiVitro® NK Cell SF Medium Supplement, and OptiVitro® Cytokine III. All of these components are serum-free, xeno-free, and have been manufactured in strict compliance with GMP regulations. This Kit can be combined with OptiVitro® NK Cell Expansion Serum-free Kit P01 (NE000-N02#) to facilitate and support the expansion of NK cells in vitro.

Contents and storage

Catalog No.	Product name	Amount	Storage	Shelf life[1]
NE000-N032	**OptiVitro® NK Cell Expansion Serum-free Basic Kit P01**	**1 kit**	-	-
BA0092	OptiVitro® NK Cell Basal SF Medium P01	1000 mL	2-8 ℃ Protect from light	12 months
BA0102	OptiVitro® NK Cell SF Medium Supplement	8 mL	2-8 ℃ Protect from light	18 months
BA0132	OptiVitro® Cytokine III	310 µL	-20 ℃	12 months
NE000-N031	**OptiVitro® NK Cell Expansion Serum-free Basic Kit P01**	**1 kit**	-	-
BA0091	OptiVitro® NK Cell Basal SF Medium P01	500 mL	2-8 ℃ Protect from light	12 months
BA0101	OptiVitro® NK Cell SF Medium Supplement	4 mL	2-8 ℃ Protect from light	18 months
BA0131	OptiVitro® Cytokine III	155 µL	-20 ℃	12 months
NE000-N031S	**OptiVitro® NK Cell Expansion Kit Basic P01 (Sample)**	**1 kit**	-	-
BA0091S	OptiVitro® NK Cell Basal SF Medium P01 (Sample)	100 mL	2-8 ℃ Protect from light	12 months
BA0101S	OptiVitro® NK Cell SF Medium Supplement (Sample)	0.8 mL	2-8 ℃ Protect from light	18 months
BA0131S	OptiVitro® Cytokine III (Sample)	31 µL	-20 ℃	12 months

[1] The Shelf-Life may be extended after strict validation by QC.

Instructions for use

Prepare media

1. Place OptiVitro® NK Cell Basal SF Medium P01 and OptiVitro® NK Cell SF Medium Supplement under a sterile laminar flow hood.
2. Add 4 mL/8 mL OptiVitro® NK Cell SF Medium Supplement to 500 mL/1000 mL OptiVitro® NK Cell Basal SF Medium P01.
3. Tighten and mix the complete OptiVitro® NK Cell Basal SF Medium P01 thoroughly.

Note: It is recommended to use complete OptiVitro® NK Cell Basal SF Medium P01 within four weeks after mixed.

4. To prepare the complete medium (short name: "NK-SFM"), add 155 µL/310 µL of OptiVitro® Cytokine III to 500 mL/1000 mL of the previously prepared OptiVitro® NK Cell Expansion Serum-free Medium P01.

Note:

1) The complete medium (the short name is 'NK-SFM' in the following protocol) is stable for 3 weeks when stored at 2-8℃ in the dark.

2) OptiVitro® Cytokine III can be aliquoted for small volume culture use but should be limited to three freeze-thaw cycles.

Culture NK cells from PBMCs

OptiVitro® NK Cell Expansion Serum-free Kit P01 is designed for culturing NK cells from peripheral blood mononuclear cells (PBMCs), cord blood mononuclear cells (CB-MNCs), or NK cells derived from iPS cells. It can be used together with OptiVitro® NK Cell Expansion Serum-free Kit P01 (NE000-N02#) for feeder-free NK cell culture systems. The kit does not necessarily require serum or serum replacement, but supplementing with heat-inactivated autologous plasma, serum replacement, or human AB serum can increase cell expansion folds. This protocol outlines the procedures for culturing NK cells from PBMCs, starting with a T75 flask as an example.

1. Thaw OptiVitro® Cytokine I (OptiVitro® NK Cell Expansion Serum-free Kit P01, NE000-N02#) at room temperature one day before NK cell activation. Add 45 µL OptiVitro® Cytokine I to 15 mL sterile DPBS and mix well. Transfer the mixed liquid to a sterile T75 flask, shaking it slightly to ensure the liquid covers the bottom of the flask. Store the flask at 2-8℃ overnight.
2. Prepare fresh PBMCs following standard PBMC separation protocols or quickly thaw (<1 minute) frozen vials of PBMC cells in a 37°C water bath.

3. If using fresh PBMCs, wash them with sterile DPBS and use them directly. If using frozen cells, thaw them one day before NK cell activation, place them at a concentration of around 2×10^6 cells/mL in complete OptiVitro® NK Cell Expansion Serum-free Medium P01 without extra cytokines, and incubate them in a humidified 37℃ incubator with an atmosphere of 5% CO_2 for 16-24 h.
4. It is optional to sort NK cells using magnetic beads with antibodies before the activation.
5. Centrifuge cells at 400×g for 10 minutes and discard the supernatant.
6. Equilibrate the T75 flask coated with OptiVitro® Cytokine I (prepared in step 1) at room temperature and remove the liquid.
7. Equilibrate complete OptiVitro® NK Cell Expansion Serum-free Medium P01 to room temperature before use. Resuspend the PBMCs at a concentration of $2.0\text{-}2.5\times10^6$ cells/mL in 15 mL of complete OptiVitro® NK Cell Expansion Serum-free Medium P01 with OptiVitro® Cytokine III (NK-SFM) supplemented with 10% heat-inactivated autologous plasma.
8. Transfer the cells (from step 7) to the T75 flask (from step 6), add 150 µL of OptiVitro® Cytokine II to the medium, and shake it slightly. Incubate the cells in a humidified 37℃ incubator with an atmosphere of 5% CO_2.
9. On Day 3 after NK cell activation, feed the cells with 15 mL of NK-SFM supplemented with 10% heat-inactivated autologous plasma.
10. On Day 5 after NK cell activation, feed the cells and adjust the cell concentration to $1.0\text{-}1.5\times10^6$ cells/mL with NK-SFM supplemented with 5% heat-inactivated autologous plasma.
11. From Day 7 after NK cell activation, feed the cells and adjust the cell concentration to $1.0\text{-}1.5\times10^6$ cells/mL with NK-SFM supplemented with 1% heat-inactivated autologous plasma every 2-3 days. The cells can be transferred to bioreactors for further expansion at around Day 9-11 after NK cell activation.

Note: If culturing NK cells from CB-MNCs, it is recommended to use freshly prepared CB-MNCs instead of frozen ones to achieve higher expansion folds.