

ExCell Bio

OptiVitro[®] NK Cell Expansion Serum-free Medium P01

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

User Manual

Catalog Number NE000-N012
NE000-N011
NE000-N011S



Product description

OptiVibro® NK Cell Expansion Serum-free Medium P01 is a serum-free, xeno-free medium that 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 medium is composed of two components: OptiVibro® NK Cell Basal SF Medium P01 and OptiVibro® NK Cell SF Medium Supplement, both components are serum-free, xeno-free, and have been manufactured in strict compliance with GMP regulations. This medium can be combined with OptiVibro® 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-N012	OptiVibro® NK Cell Expansion Serum-free Medium P01	1 kit	-	-
BA0092	OptiVibro® NK Cell Basal SF Medium P01	1000 mL	2-8 °C Protect from light	12 months
BA0102	OptiVibro® NK Cell SF Medium Supplement	8 mL	2-8 °C Protect from light	18 months
NE000-N011	OptiVibro® NK Cell Expansion Serum-free Medium P01	1 kit	-	-
BA0091	OptiVibro® NK Cell Basal SF Medium P01	500 mL	2-8 °C Protect from light	12 months
BA0101	OptiVibro® NK Cell SF Medium Supplement	4 mL	2-8 °C Protect from light	18 months
NE000-N011S	OptiVibro® NK Cell Expansion Serum-free Medium P01 (Sample)	1 kit	-	-
BA0091S	OptiVibro® NK Cell Basal SF Medium P01 (Sample)	100 mL	2-8 °C Protect from light	12 months
BA0101S	OptiVibro® NK Cell SF Medium Supplement (Sample)	0.8 mL	2-8 °C Protect from light	18 months

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

| Instructions for use

Prepare media

1. Place OptiVibro® NK Cell Basal SF Medium P01 and OptiVibro® NK Cell SF Medium Supplement under a sterile laminar flow hood.
2. Add 4 mL/8 mL OptiVibro® NK Cell SF Medium Supplement to 500 mL/1000 mL OptiVibro® NK Cell Basal SF Medium P01.
3. Tighten and mix the OptiVibro® NK Cell Basal SF Medium P01 thoroughly.
4. The complete OptiVibro® NK Cell Basal SF Medium P01 can be supplemented with cytokines such as IL-2, IL-15 to support NK cell expansion.

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

Culture NK cells from PBMCs

OptiVibro® NK Cell Expansion Serum-free Medium 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 OptiVibro® NK Cell Expansion Serum-free Kit P01 (NE000-N02#) for feeder-free NK cell culture systems. The medium 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. The protocol lists the procedures of the feeder-free culture of NK cells from PBMCs as an example.

1. Prepare fresh PBMCs following standard PBMC separation protocols or quickly thaw (<1 minute) frozen vials of PBMCs in a 37°C water bath.
2. 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 OptiVibro® NK Cell Expansion Serum-free Medium P01 without extra cytokines, and incubate them in a humidified 37°C incubator with an atmosphere of 5% CO₂ for 16-24 h.
3. It is optional to sort NK cells using magnetic beads with antibodies before the activation.
4. Centrifuge cells at 400×g for 10 minutes and discard the supernatant.
5. Equilibrate complete OptiVibro® NK Cell Expansion Serum-free Medium P01 to room temperature before use. Resuspend PBMCs at a concentration of $2.0\text{-}2.5 \times 10^6$ cells/mL in complete OptiVibro® NK Cell Expansion Serum-free Medium P01 supplemented with 10% heat-inactivated autologous plasma and cytokines such as

IL-2, IL-15 for NK cell expansion.

6. Transfer the cells to culture plates pre-coated with antibodies/cytokines for activating NK cells to initiate the culture. Other cytokines or chemicals for NK cell activation should be added at this time.
7. Incubate the cells in a humidified 37°C incubator with atmosphere of 5% CO₂.
8. On Day 3 after NK cell activation, feed the cells with same volume of complete OptiVibro® NK Cell Expansion Serum-free Medium P01 supplemented with 10% heat-inactivated autologous plasma and cytokines.
9. On Day 5 after NK cell activation, feed the cells and adjust the cell concentration to 1.0-1.5×10⁶ cells/mL with complete OptiVibro® NK Cell Expansion Serum-free Medium P01 supplemented 5% heat-inactivated autologous plasma and cytokines.
10. From Day 7 after NK cell activation, feed the cells and adjust the cell concentration to 1.0-1.5×10⁶ cells/mL with complete OptiVibro® NK Cell Expansion Serum-free Medium P01 supplemented 5% heat-inactivated autologous plasma and cytokines every 2-3 days. The cells can be transferred to bioreactors for further expansion at around Day 9-11 after NK cell activation.