

# ExCell Bio

## **OptiVitro<sup>®</sup> NK Cell Expansion Serum-free Medium NE01 (phenol red-free)**

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

### **User Manual**

Catalog Number NE000-N042  
NE000-N041  
NE000-N041S



## | Product description

The OptiVibro® NK Cell Expansion Serum-free Medium NE01 (phenol red-free) 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 two main components: OptiVibro® NK Cell Basal SF Medium NE01 (phenol red-free) and OptiVibro® NK Cell SF Medium Supplement. All of these components are serum-free, xeno-free, and have been manufactured in strict compliance with GMP regulations.

## | Contents and storage

Catalog No.	Product name	Amount	Storage	Shelf life <sup>[1]</sup>
<b>NE000-N042</b>	<b>OptiVibro® NK Cell Expansion Serum-free Medium NE01 (phenol red-free)</b>	<b>1 kit</b>	-	-
BA0142	OptiVibro® NK Cell Basal SF Medium NE01 (phenol red-free)	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
<b>NE000-N041</b>	<b>OptiVibro® NK Cell Expansion Serum-free Medium NE01 (phenol red-free)</b>	<b>1 kit</b>	-	-
BA0141	OptiVibro® NK Cell Basal SF Medium NE01 (phenol red-free)	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
<b>NE000-N041S</b>	<b>OptiVibro® NK Cell Expansion Serum-free Medium NE01 (phenol red-free) (Sample)</b>	<b>1 kit</b>	-	-
BA0141S	OptiVibro® NK Cell Basal SF Medium NE01 (phenol red-free) (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

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

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## | Instructions for use

### **Prepare media**

1. Place OptiVibro® NK Cell Basal SF Medium NE01 (phenol red-free) 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 NE01 (phenol red-free).
3. Tighten and mix the complete OptiVibro® NK Cell Expansion Serum-free Medium NE01 (phenol red-free) thoroughly.
4. Complete OptiVibro® NK Cell Expansion Serum-free Medium NE01 (phenol red-free) may 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 Expansion Serum-free Medium NE01 (phenol red-free) within four weeks after mixed.

### **Culture NK cells from PBMCs**

The OptiVibro® NK Cell Expansion Serum-free Medium NE01 (phenol red-free) 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 the OptiVibro® NK Cell Expansion Serum-free Kit NE01 (phenol red-free) (NE000-N05#) 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 lists the procedures of the feeder-free culture of NK cells from PBMCs as example.

1. Prepare fresh PBMCs following standard PBMC separation protocols or quickly thaw (<1 minute) frozen vials of PBMC cells 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  $2 \times 10^6$  cells/mL in complete OptiVibro® NK Cell Expansion Serum-free Medium NE01 (phenol red-free) without extra cytokines, and incubate them in a humidified 37°C incubator with an atmosphere of 5% CO<sub>2</sub> 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 NE01 (phenol red-free) to room

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temperature before use. Resuspend PBMCs at a concentration of  $2.0-2.5 \times 10^6$  cells/mL in complete OptiViro® NK Cell Expansion Serum-free Medium NE01 (phenol red-free) 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% CO<sub>2</sub> in air.
8. On Day 3 after NK cell activation, feed the cells with same volume of OptiViro® NK Cell Expansion Serum-free Medium NE01 (phenol red-free) 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  $1.0-1.5 \times 10^6$  cells/mL with complete OptiViro® NK Cell Expansion Serum-free Medium NE01 (phenol red-free) 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  $1.0-1.5 \times 10^6$  cells/mL with complete OptiViro® NK Cell Expansion Serum-free Medium NE01 (phenol red-free) supplemented 1% 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.