

## SUPERCULTURE N500 Serum-Free Medium for NK Cells

Cat#: 6113031

### [Product Name]

N500 Serum-Free Medium for NK cells

### [Product Description]

The N500 Serum-Free Medium for NK cells is a special medium for in vitro culture of NK cells developed by Dakewe, and is recommended to be used with the NK Cell Stimulator (Cat#: XC0031A) and the NK Cell Activator (Cat#: XC0032A), or can be used through other in vitro culture schemes for NK cells. This product is a chemically defined medium, all the compositions of which are produced from medicinal/cell culture grade materials. This product contains no animal component or serum or any other protein component except albumin, transferrin and insulin and can effectively avoid the influence of serum qualitative variation, serum components and exogenous components on experimental research. The optimized formula of this product can significantly improve the proportion, the viability and the killing rate of NK cells in cells cultured in vitro.

### [Model & Specification]

Product Name	Cat. No.	Size
N500 Serum-Free Medium for NK Cells	6113031	1000 mL

### [Storage Conditions and Validity Period]

Keep away from light at 2~8°C, valid for 12 months.

### [Directions for Use]

The N500 Serum-Free Medium for NK cells supports the high-density suspension culture of lymphocytes. In a static culture container, to ensure good gas exchange of the culture system, the recommended depth of the medium is not more than 1.5 cm. The medium supports the high-density culture of NK cells in a bioreactor, and **the optimization of the culture procedure is determined by users based on experience.**

The N500 Serum-Free Medium for NK cells supports the serum-free culture of NK cells, and the addition of autologous plasma, human AB serum, FBS or serum substitutes can significantly improve the cell state and increase the cell proliferation rate. Autologous plasma or Cell Culture Supplemental Mix (Cat#: 6122011/6122012) is recommended to obtain the optimal culture effect.

### Culture Example

1. Place the N500 Serum-Free Medium for NK cells at room temperature for equilibrium.

The following operations shall be in an aseptic condition:

2. Add 500  $\mu$ L of **NK Cell Activator** (Cat#: XC0032A) per liter of N500 Serum-Free Medium for NK cells to prepare an **Amplification Medium for NK cells**.

3. Separate lymphocytes of human peripheral blood in an aseptic condition (it is recommended to use a human lymphocyte separation tube/separation medium, Cat#: 7922021 / 7912011).

4. Heat-inactivated autologous plasma: obtain plasma by separation, heat-treat the plasma at 56°C for 30 min, centrifuge the plasma at 1000 g for 10 min, and then extract the supernatant to obtain heat-inactivated autologous plasma.

5. On the zeroth day, inoculate PBMCs obtained by separation to a T75 or T175 cell culture bottle with the **Amplification Medium for NK cells** (containing 10% of heat-inactivated autologous plasma) at a concentration of  $2.5\sim 3.0\times 10^6$  cells/mL, add a NK Cell Stimulator (100 $\times$ ) (Cat#: XC0031A), and culture in a 5% CO<sub>2</sub> incubator at 37°C.

6. On the third day, supplement fresh Amplification Medium for NK cells (containing 10% of

heat-inactivated autologous plasma). The volume ratio of the original medium to the supplemented fresh Amplification Medium for NK cells is 1:2.

(For example, for 10 mL of original medium, supplement 20 mL of fresh Amplification Medium for NK cells.)

7. From the fifth day, sample every two days to calculate the cell concentration, supplement fresh **Amplification Medium for NK cells** (containing 5% of heat-inactivated autologous plasma) based on the calculation result, and adjust the cell concentration to  $0.8 \sim 1 \times 10^6$  cells/mL.

(The cell density can be adjusted according to changes of medium color. When cells are passaged, the passage density can be adjusted to  $0.8 \sim 0.9 \times 10^6$  cells/mL if the medium is yellow, and can be adjusted to  $1 \times 10^6$  cells/mL if the medium is red.)

8. On the seventh day, change a bottle with a larger volume or transfer into a cell culture bag (it is recommended to use Cell Culture Bags, Cat#: 6071011/6071012) according to the volume of the cell culture suspension. The maximum culture volume of a T75 culture bottle is 40 mL, and the maximum culture volume of a T175 culture bottle is 200 mL. When the volume of the medium is more than 200 mL, transfer the cell culture suspension into a cell culture bag.









9. After the seventh day, the content of the heat-inactivated autologous plasma in the supplemented fresh Amplification Medium for NK cells can be reduced to 1%.

10. Cell proliferation and cell surface marker detection.

#### [Notes]

- During blood collection, heparin anticoagulant can be used, but EDTA anticoagulant cannot be used.
- The blood collection volume can be estimated based on  $1 \times 10^6$  PBMCs per milliliter of peripheral blood.
- Before use, the medium should be equilibrated at room temperature, or the expected amount should be prewarmed to 37°C. Do not place the whole bottle of medium at 37°C for repeated prewarming.
- If the temperature of the medium is too low or the cell density is too high, flocculated cells may appear, and the cell viability is reduced.
- Cell passage shall be carried out gently to avoid mechanical damages to cells.
- The Amplification Medium for NK cells is valid for three weeks.
- **The culture example is only for reference, and the optimization of the culture procedure is determined by users based on experience.**

#### [Description of Product Symbol]

Product Symbol	Description	Product Symbol	Description
	Catalogue number		Batch code
	Date of manufacture		Temperature limit
	Use-by date		Consult instructions for use
	Manufacturer		Keep away from sunlight

**[Related Products]**

Product Name	Cat. No.	Size
Natural Killer Cells Induction Culture Kit 2.0	6813533	3 L/kit
	6813532	2 L/kit
	6813531	1 L/kit
NK Cell Activator	XC0032A	500 µL
NK Cell Stimulator	XC0031A	500 µL
Lymphocyte Separation Tube for Human Peripheral Blood	7922021	50 mL × 25 vials
Human Lymphocyte Separation Kit (GMP)	7825121	60 test
Human Lymphocyte Separation Kit	7125121	60 tests
Density Reagent	7912011	250 mL
Human Lymphocyte Separation Medium	7111011	100 mL
Cell Culture Supplemental Mix	6122012	25 mL
	6122011	250 mL
Cell Culture Bags	6071011	640 cm <sup>2</sup> , 10 bags/package
	6071012	640 cm <sup>2</sup> , 10 bags/package × 10, 100 bags/carton

**[Instruction Revision Date]**

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