

Biosci™ Natural Killer Cells Induction Culture Kit 2.0

Instructions for Use

Cat#: 6813531/6813532/6813533

Product Description

The SuperCulture™ Natural Killer Cells Induction Culture Kit 2.0 is a culture kit for in vitro culture of NK cells developed in GMP confirming processes by Dakewe, which contains N500 Serum Free Medium for NK Cells, NK cell activator and NK cell stimulator. This product is a chemically defined, 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.

Packing Specification

Cat.	Name	Specification	Components	
6813531	Natural Killer Cells Induction Culture Kit 2.0	3L/kit	NK cell stimulator	150μL
			NK cell activator	500μL×3
			N500 Serum Free Medium	1L×3
6813532		2L/kit	NK cell stimulator	100μL
			NK cell activator	500μL×2
			N500 Serum Free Medium	1L×2
6813533		1L/kit	NK cell stimulator	50μL
			NK cell activator	500μL
			N500 Serum Free Medium	1L

How to Use

The SuperCulture™ Natural Killer Cells Induction Culture Kit 2.0 supports the high-density suspension culture of NK cells. 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 SuperCulture™ Natural Killer Cells Induction Culture Kit 2.0 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 SuperGrow™ cell culture additive (Cat#: 6122011/6122012) is recommended to obtain the optimal culture effect.

Culture Example

1. Place the **SuperCulture™ N500 serum free medium for NK cells** at room temperature for equilibrium.

The following operations shall be in an aseptic condition:

2. Add 500 uL of **NK cell activator** per liter of SuperCulture™ 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 Biosci™ human lymphocyte separation tube/separation medium, Cat#: 7912011/7922021).

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×)**, and culture in a 5% CO₂ 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 1×10^6 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 a Biosci™ cell culture bag, 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.

Preservation Method

Keep NK cell stimulator and NK cell activator at -25~ -10°C, valid for 12 months.

Keep N500 medium away from light at 2~8°C, valid for 12 months.

Notes

- During blood collection, heparin or sodium citrate anticoagulant can be used, but EDTA anticoagulant cannot be used.
- The blood collection volume can be estimated based on 1×10^6 PBMCs per milliliter of peripheral blood.
- Place the medium at room temperature for equilibrium or extract an estimated amount, and reheat to 37°C.
- 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.
- Use up the NK cell stimulator and NK cell activator at one time, and it shall not be frozen and thawed repeatedly, and shall be used within the validity of the product. After the complete medium is prepared by mixing, it is recommended to be stored away from light at 2°C to 8°C and used up within two weeks, and attention shall be paid to operate in sterile conditions.
- Research use only.
- **The culture example is only for reference, and the optimization of the culture procedure is determined by users based on experience.**