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Natural Killer Cells Induction Culture Kit

Cat. #: C0127 Size: 1 L

Introduction

The Natural Killer (NK) Cells Induction Culture Kit is a culture kit for *in vitro* culture of NK cells developed in GMP confirming processes, 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*.

Package Information

Component	C0127
N500 Serum-Free Medium	1 L
NK cell activator	500 μL
NK cell stimulator	50 μL

Storage

NK cell stimulator and NK cell activator at -25°C~-10°C; N500 Serum-Free Medium at 2~ 8°C and protect from light.

Applications

The NK Cells Induction Culture Kit 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 kit 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 additive is recommended to obtain the optimal culture effect.

Protocol

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 μL of NK cell activator 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 #: C0130).

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 $2.5\sim3.0\times10^6$ cells/mL, add a NK cell stimulator (100×) , and culture in a 5% CO_2 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 $0.8\sim1\times10^6$ 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 $0.8\sim0.9\times10^6$ 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 according to the volume of the cellculture 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.

Note:

1. During blood collection, heparin or sodium citrate anticoagulant can be used, but EDTA anticoagulant cannot be used.

2. The blood collection volume can be estimated based on 1×10^6 PBMCs per milliliter of peripheral blood.

3. Place the medium at room temperature for equilibrium or extract an estimated amount, and reheat to 37°C.

4. 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.

5. Cell passage shall be carried out gently to avoid mechanical damages to cells.

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6. The amplification medium for NK cells is valid for three weeks.

7. 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.