

# NK-MAX Purification Factor

## Product Manual

### I. Product Introduction

**NK-MAX Purification Factor** is a reagent used in the separation process of fresh peripheral blood/umbilical cord blood (mononuclear cells), primarily to increase the proportion of CD3-CD56+ cells in the isolated mononuclear cells. When used in combination with the Shownin Biotech NK Expansion Kit (RP03030), it ensures a **very high purity of the final expanded NK cells (CD3-CD56+ expression rate exceeding 95%)** and less than 1% CD3+ cells.

### II. Product Information

Table 1: Product Description of NK-MAX Purification Factor

Product Information	Cat.No.	Amount	Storage
NK-MAX Purification Factor	SN-03-0050	0.625ml	Store at 4°C Transport at 2-8°C

\* The NK-MAX Purification Factor may form precipitates during transportation and storage. Before use, shake well to re-suspend the solution.

### III. Reagents and Materials

Table 2: Recommended Reagent & Material

Reagent & Material	Brand (e.g.)	Cat.No.(e.g.)
NK-MAX Purification Factor	Shownin Biotech	SN-03-0050
DPBS	Gibco	C14190500BT

### IV. NK-MAX Purification Factor Application for Mononuclear Cell Preparation

**4.1. Mononuclear Cell Preparation:** Mononuclear cells are typically derived from peripheral blood and umbilical cord blood. They are classified into two forms: fresh sample separation and thawing of frozen samples. Please refer to the corresponding operational steps based on the actual situation.

Tips:

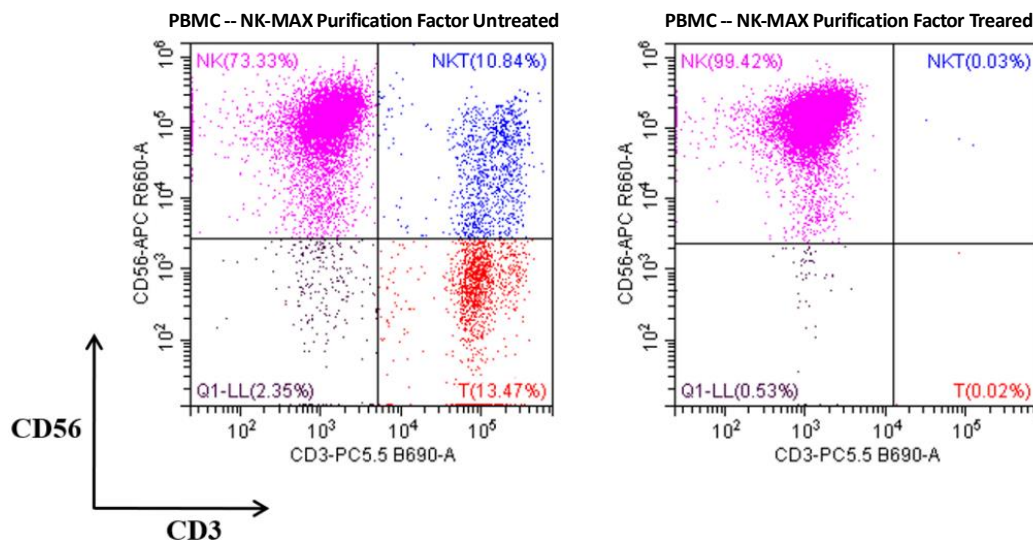
- (1) To avoid excessive anticoagulant affecting the use of autologous plasma, the anticoagulant ratio in cord blood should be kept below 30%.
- (2) For blood collection, we recommend using heparin sodium anticoagulant vacuum blood collection tubes. Do not use EDTA anticoagulant vacuum tubes, as EDTA can affect NK cell activation and expansion.
- (3) For samples separated using autologous plasma, the cell density should not exceed  $5 \times 10^7$  cells/mL when using the NK-MAX Purification Factor.

#### 4.2. Fresh Sample Separation (Methods for Peripheral Blood and Umbilical Cord Blood are Similar)

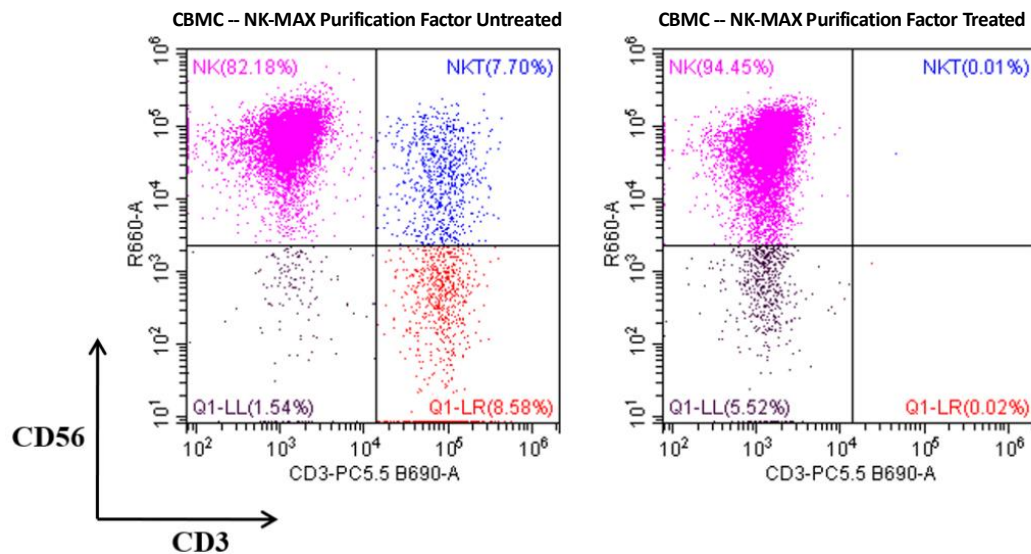
**4.2.1 Autologous Plasma Separation (Optional):** Centrifuge fresh blood at 900×g for 20 minutes (set acceleration/deceleration to the slowest). After centrifugation, carefully aspirate the upper pale-yellow plasma into a 50mL centrifuge tube (the remaining blood cell layer can be used for PBMC separation). Place the plasma in a 56°C water bath for 30 minutes to inactivate, then centrifuge at 1200×g for 10 minutes to remove any precipitate. Transfer the inactivated plasma into a new 50mL centrifuge tube and store at 4°C for future use.

**4.2.2 Using the NK-MAX Purification Factor:** Add **12.5 $\mu$ L** of NK-MAX Purification Factor per mL of fresh blood volume (before autologous plasma separation) into the centrifuge tube containing the blood sample. Mix thoroughly using a pipette and incubate at room temperature for 20 minutes.

**4.2.3 PBMC Separation:** After removing the plasma as described in 4.2.1, dilute the remaining blood cell layer with saline in a 1:1 ratio and mix thoroughly. Add this mixture to a centrifuge tube containing Ficoll (avoid disturbing the liquid interface). Centrifuge at 900 $\times$ g for 30 minutes. Carefully aspirate the white interface layer into a new centrifuge tube. Wash the cells with cell wash buffer (DPBS + 2% hPL or autologous plasma) and centrifuge at 400 $\times$ g for 10 minutes, discard the supernatant. Repeat steps 1-2 until Ficoll is completely removed. The PBMC pellet can be used for direct activation and culture or stored for future use (refer to the lymphocyte separation reagent instructions for different protocols). The isolated PBMCs can be used for subsequent NK activation and expansion; for detailed expansion protocols, refer to the NK Expansion Kit (RP03030) manual.



**Figure 1: Peripheral Blood (PBMC) - Comparison of NK Cell Purity between NK-MAX Purification Factor Treated and Untreated Groups**



**Figure 2: Umbilical Cord Blood (CBMC) - Comparison of NK Cell Purity between NK-MAX Purification Factor Treated and Untreated Groups**