ncKnight® NK Expansion Factor Kit
Product Manual

I. Product Introduction

ncKnight® NK Expansion Factor Kit is a specialized culture system independently developed by Shownin Biotech Co., Ltd., designed for the efficient expansion of human natural killer (NK) cells. This **pure factor-based system** supports the expansion of NK cells derived from human peripheral blood, achieving a substantial increase of **3000~6000 times over a 13~15 day cultivation period (based on 10% NK cells in PBMC)**. The final expanded NK cells exhibit high purity **(CD3- CD56+ expression rates exceeding 80%~95%)**.

II. Product Information

Table 1: Product Description of ncKnight® NK Expansion Factor Kit

Product Information	Cat.No.	Amount	Quantity	Storage
ncKnight® NK Expansion Factor Kit Contains:	**SN-03-0030**	**1 Kit (2L)**	**1**	
ncKnight® NK Expansion Factor A	SN-03-0031	135 µL	1	Store at -20°C ~ -80°C Transport on dry ice
ncKnight® NK Expansion Factor B	SN-03-0032	60 µL	1	
ncKnight® NK Expansion Medium	SN-03-0020	1L/bottle	2	Store at 4°C Transport at 2-8°C

III. Reagents and Materials

Table 2: Recommended Reagent & Material

Reagent & Material	Brand (e.g.)	Cat.No.(e.g.)
ncKnight® NK Expansion Factor Kit	Shownin Biotech	SN-03-0030
Recombinant Human Interleukin-2 (for injection)	Gold Seal	State Pharmaceutical Approval No. S10970058
Human Platelet Lysate (PLT)	NA	NA
DPBS	Gibco	C14190500BT
Physiological Saline	Anhui Shuanghe Pharmaceutical	State Pharmaceutical Approval No. H34023608
6-Well Plate	Nunc	140675
T182 Cell Culture Flask	Guangzhou Jiete	TCF102600
Lymphocyte Culture Bag (0.2-1.8L)	Takara	GT-T610(A)

IV. Mononuclear Cell Preparation

4.1. Mononuclear Cell Preparation: Mononuclear cells typically come from peripheral blood and can be separated from fresh samples or thawed from frozen samples. Please refer to the corresponding operation steps based on the sample type.

4.2. Fresh Peripheral Blood Separation

4.2.1 Autologous Plasma Separation: 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 PBMC Isolation: 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 800×g for 25 minutes, then carefully aspirate the middle white layer (PBMCs). Wash the cells twice with saline or DPBS and count them. Next, centrifuge the cells at 400×g for 10 minutes and discard the supernatant. The resulting PBMC pellet can either be directly used for activation and culture (refer to Step 5) or stored as needed. (For different lymphocyte separation media, follow the corresponding instructions in the respective manual.)

4.3. Frozen Peripheral Blood Sample Treatment

DAY -1: For the preparation of frozen mononuclear cells, they should be thawed and equilibrated 24 hours in advance. Thaw at least 30 million PBMCs. After thawing the cells in a 37°C water bath, transfer them to a biosafety cabinet. Transfer the cell suspension to a sterile centrifuge tube and slowly add 20 mL of pre-warmed NK expansion complete medium, mixing gently while adding. After complete addition, mix gently, centrifuge at 300×g for 5 minutes to remove the supernatant. Resuspend the cells in NK expansion complete medium (maintaining PBMC density at 2×10^6/mL) and inoculate into a T75 culture flask. Culture overnight in a 37°C incubator.

V. Activation and Expansion Culture

5.1 NK Expansion Complete Medium Preparation: Prepare NK expansion complete medium + IL-2 (200 IU/mL).

5.2 Activation Medium Preparation: Add 10mL of NK expansion complete medium + 5% autologous plasma/PLT to a 50mL centrifuge tube. Add thawed NK expansion factors A and B. Mix thoroughly with a pipette.

5.3 DAY0 - NK Cell Activation: For fresh PBMCs, take 5×10^6 ~ 10×10^6 viable cells directly, or for thawed PBMCs, after 24 hours of equilibration and counting, take 5×10^6 ~ 10×10^6 viable cells. Add 5mL activation medium for 5×10^6 cells, or 10mL for 10×10^6 cells. Mix well and transfer the cell suspension to T25/T75 flasks. Place the flasks in a 37°C, 5% CO_2 incubator and culture for 3 days.

5.4 DAY3 - Media Addition: Perform a media addition by doubling the initial volume: for 5×10^6 PBMCs, add 5mL NK expansion complete medium + 5% autologous plasma/PLT; for 10×10^6 PBMCs, add 10mL. Place in a 37°C, 5% CO_2 incubator.

5.5 DAY4~7 - Daily Media Addition: Add NK expansion complete medium + 5% autologous plasma/PLT according to cell density or color. Ensure the cell density remains between 0.7×10^6 cells/mL and 1.5×10^6 cells/mL. If the total volume exceeds 50mL, transfer the cells to T182/T175 flasks for continued culture.

5.6 DAY8~12 - Media Addition: Add NK expansion complete medium + 2% autologous plasma/PLT. Maintain cell density

between 0.7×10^6 cells/mL and 1.5×10^6 cells/mL. If the total volume exceeds 200mL, transfer to a cell culture bag for continued culture (around Day 8/9).

5.7 DAY13~16 - Harvest Cells: Cells are generally harvested between Day 13 and Day 16 for optimal results.

Table 3: NK Culture Process Reference Volume of Medium*

Time	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D12	D13~D16
Volume (mL)	10	10	20	40	80	160	320	640	1000	1500	2000	2000
Operation	Activation	--	--	Media Addition	Medium Addition	Medium Addition	Medium Addition	Medium Addition Bag Transfer	Medium Addition	Medium Addition	Medium Addition	Medium Addition
Culture Vessel	T75			T182				Lymphocyte Culture Bag				

***This table is for reference only, based on an initial 10×10^6 viable cells. Due to individual sample differences, the volume of medium may fluctuate. After observing and analyzing the growth condition of NK cells, adjust the volume based on the optimal cell growth density.**