NK Amplification Kit

Product Manual

I. Product Introduction

NK Amplification Kit is designed for the expansion and culture of human Natural Killer (NK) cells. It can be used with mainstream immune cell culture media to efficiently expand various human NK cells (such as peripheral blood-derived and umbilical cord blood-derived NK cells). NK cell expansion can achieve 6000-10000 times after 11-13 days of culture, assuming 10% of NK cells in peripheral blood mononuclear cells (PBMCs), with a high purity of NK cells (CD3-CD56+ expression rate >90%).

II. Product Information

Table 1: NK Amplification Kit Product Descriptions

Product Information	Cat.No.	Amount	Storage
NK Amplification Kit Contains：	**RP03030**	**1 Kit**	**Liquid Nitrogen Storage Dry Ice Transportation**
NK Amplification Kit A	RP03030-A	1 mL	
NK Amplification Kit B	RP03030-B	4 mL	

III. Reagents and Materials

Table 2: Recommended Supporting Reagents & Materials

Reagents & Materials	Brand (e.g.)	Cat.No. (e.g.)
NK Amplification Kit	Shownin Biotech	RP03030
Lymphocyte serum-free medium	CORNING	88-581-CM
Recombinant Human Interleukin-2 for Injection	SL PHARM	Xinjier
Human platelet lysate (PLT)	NA	NA
T75 Culture Flasks	Thermo Fisher	156499
T175 Culture Flask	Thermo Fisher	159910
Lymphocyte culture bag (0.2-1.8L)	Takara	GT-T610(A)

IV. Preparation of Peripheral Blood Mononuclear Cells (PBMCs)

4.1 PBMC Source and Preparation：PBMCs are typically isolated from peripheral blood or umbilical cord blood. The process may involve separation from fresh samples or recovery from cryopreserved samples. Please follow the appropriate procedure based on the sample type；

4.2 Isolation from Fresh Samples (Peripheral Blood and Umbilical Cord Blood)

Note: (1) To prevent excess anticoagulant from interfering with autologous plasma use, the proportion of anticoagulant in umbilical cord blood should be kept below 30%.

(2) For blood collection, it is recommended to use vacuum blood collection tubes with heparin sodium as the anticoagulant. Do not use EDTA tubes, as EDTA may impair NK cell activation and expansion.

4.2.1 Autologous Plasma Preparation：Centrifuge fresh blood at 800 ×g for 25 minutes (with the slowest acceleration/deceleration settings). After centrifugation, collect the upper light-yellow plasma layer into a 50 mL centrifuge tube (the remaining blood cell layer is used for PBMC isolation).
Inactivate the plasma at 56°C in a water bath for 30 minutes. Then centrifuge at 1200 ×g for 10 minutes to remove any precipitate. Transfer the inactivated plasma to a new 50 mL tube and store at 4°C for later use.

4.2.2 PBMC Isolation：Dilute the remaining blood cell fraction (after plasma removal) 1:1 with saline and mix gently. Carefully layer the diluted sample over Ficoll in a centrifuge tube, avoiding disruption of the interface.
Centrifuge at 900 ×g for 30 minutes. Collect the mononuclear cell layer (buffy coat), wash twice with saline, and count the cells. Centrifuge at 400 ×g for 10 minutes and aspirate the supernatant. The resulting PBMC pellet can either be used directly for activation and culture (refer to Section V), or cryopreserved as needed.(Use appropriate density gradient media and follow the manufacturer's instructions for specific reagents.)

V. NK Cell Expansion (Refer to Table 3)

5.1 Preparation of Serum-Free NK Cell Culture Medium: Mix lymphocyte serum-free medium with IL-2 to a final concentration of 200 IU/mL.

5.2 Preconditioning of Cryopreserved PBMCs: Thaw cryopreserved PBMCs in a 37°C water bath. Transfer the cell suspension to a sterile centrifuge tube in a biosafety cabinet. Slowly add 10 mL of prewarmed serum-free NK medium dropwise while gently mixing. After complete addition, mix gently, centrifuge at 300×g for 5 minutes, discard the supernatant, resuspend the pellet in serum-free NK medium, and incubate overnight at 37°C in a CO_2 incubator.

5.3 Thawing of NK Expansion Reagent A: Follow the same thawing procedure as in step 5.2. After centrifugation and supernatant removal, resuspend cells in serum-free NK medium supplemented with 5% autologous plasma (or platelet lysate [PLT] if plasma is insufficient).

Note: Reagent A is sensitive to osmotic pressure; follow the thawing protocol strictly.

Table 3: NK Cell Culture Process Reference Volumes*

Time	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12-D14
Volumetric (mL)	10	10	10	15	25	50	100	200	400	800	1000	1200	--

Operation	Add A	---	---	Medium top-up	Medium top-up	Medium top-up Transfer to a T175 flask	Medium top-up	Add B Medium top-up	Medium top-up Transfer to Bag	Medium top-up	Medium top-up	Medium top-up	Cell counting and harvest
Vessel	**T75 Flask**					**T175 Flask**			**Lymphocyte Culture Bag**				

*This table is for reference only. Due to sample variability, medium volumes may fluctuate. Culture supplementation should be adjusted according to actual cell growth and density measurements.

5.4 Day 0 - NK Cell Activation: Take 5×10^6 viable PBMCs (freshly isolated or thawed after 24-hour resuscitation), add Resuscitated NK Expansion Reagent A + NK cell serum-free medium (adjust to 10 mL) + 5% autologous plasma/PLT (0.5 mL). Mix in a T75 culture flask and incubate at 37°C with 5% CO_2 for 3 days.

5.5 Day 3 – Medium Top-up：Add 5 mL of serum-free NK medium and 0.25 mL of 5% autologous plasma/PLT.

5.6 Days 4～6 – Daily Medium Top-up：Top up with serum-free NK medium supplemented with 5% autologous plasma/PLT based on suspension color or cell density. Maintain post-top-up cell density at 0.7×10^6 to 1.5×10^6 cells/mL (recommended: 1.0×10^6 cells/mL). Once total volume exceeds 50 mL, transfer to a T175 culture flask for continued expansion.

5.7 Day 7 – NK Cell Expansion：Thaw NK Expansion Reagent B following the procedure in step 5.2. Transfer the thawed reagent into a 50 mL sterile centrifuge tube, then slowly add 27 mL of prewarmed serum-free NK medium dropwise. Resuspend the reagent in serum-free NK medium supplemented with 1% autologous plasma/PLT. Count the cells, add to the culture flask, and top up the volume to 200 mL.

5.8 Days 8～10 – Daily Medium Top-up：Continue daily top-up with serum-free NK medium supplemented with 1% autologous plasma/PLT. If the culture volume exceeds 200 mL (usually on Day 8 or 9), transfer to a culture bag for further expansion.

5.9 Days 11～14 – Medium Top-up and Harvest：Continue daily top-up. In most cases, Day 11–14 is optimal for harvesting expanded NK cells.

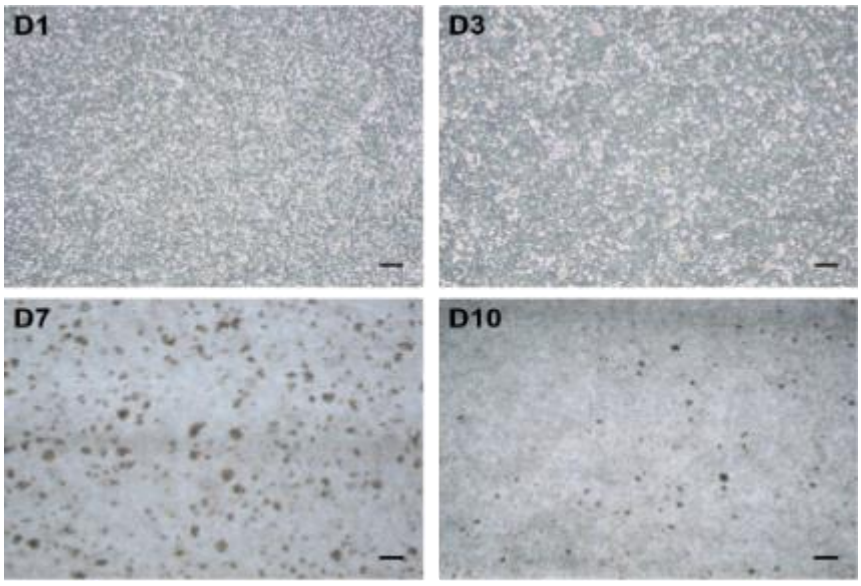

Figure 1. Morphology of PBMC-derived NK cells on Days 1, 3, 7, and 10 during expansion. Scale bar: 200 µm.

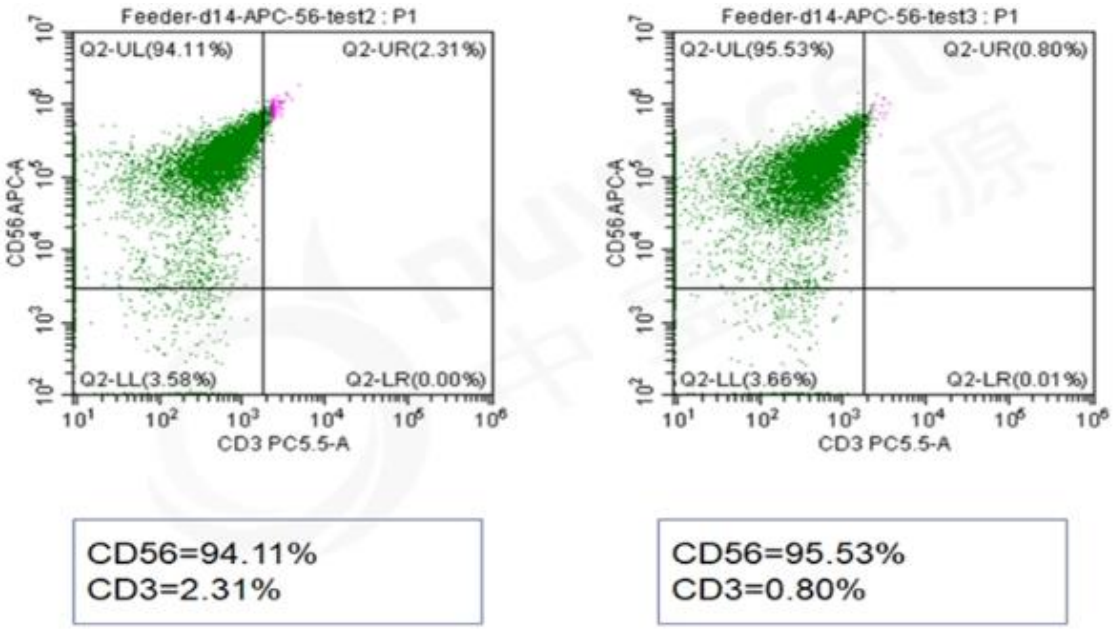

Figure 2 . Purity Test of PBNK Cells after 14 Days of Culture.
CD3- CD56+ cell ratio > 90%.

VI. Potential Problems and Solutions During NK Cell Culture

Question 1: Significant cell aggregation observed on Day 3. Is this normal, and should intervention be made?

Answer: NK cells specifically bind to NK Expansion Reagents and get activated. Macro-structurally, this results in cell aggregation, which is a normal phenomenon and indicates successful activation. Typically, by Day 7, this aggregation starts to subside, and cells begin to grow in small clusters. During this process, it is not recommended to pipette the cells; daily medium top-up is sufficient.

Question 2: What is the preferred method for cell counting?

Answer: The preferred method is using a hematology cell counter. Alternatively, Vicell (Beckman) or Countstar cell counters can be used. There may be some variability between different counting instruments, so it is recommended to choose based on practical needs.