NkVita Immune Cell Serum-Free Medium
Product Manual

I. Product Introduction

NkVita Immune Cell Serum-Free Medium is independently developed by Shownin Biotech for the expansion of human Natural Killer (NK) cells. When used in combination with the Shownin Biotech NK Expansion Kit (RP03030), it enables the efficient expansion of NK cells derived from **human peripheral blood and umbilical cord blood** (**NK cell expansion can reach 5000~10000 times in 14~15 days**, assuming 10% NK in PBMCs). The final expanded NK cells exhibit high purity (**CD3- CD56+ expression rate exceeding 90%**).

II. Product Information

Table 1: Product Description of NkVita Immune Cell Serum-Free Medium

Product Information	Cat.No.	Amount	Quantity	Storage
NkVita Immune Cell Serum-Free Medium Contains:	SN-03-0060	1 L/Kit	1	
NkVita Immune Cell Serum-Free Medium Supplement-01	SN-03-0061	100 µL/tube	1	-20°C or -80°C
NkVita Immune Cell Serum-Free Medium Supplement-02	SN-03-0062	40 mL/bottle	1	-80°C
NkVita Immune Cell Serum-Free Medium Basal Medium	SN-03-0063	1 L/bottle	1	2–8°C

III. Reagents and Materials

Table 2: Recommended Reagent & Material

Reagent & Material	Brand (e.g.)	Cat.No.(e.g.)
NkVita Immune Cell Serum-Free Medium	Shownin	SN-03-0060
NK Expansion Kit	Shownin	RP03030
Recombinant Human IL-2 for Injection	NA	NA
T75 Cell Culture Flask	NA	NA
T175 Cell Culture Flask	NA	NA
Lymphocyte Culture Bag (0.2–1.8 L)	Takara	GT-T610(A)

IV. PBMC Preparation

4.1. **PBMC Preparation:** Recommended PBMC sources include **peripheral blood.** Processing may involve fresh isolation or thawing of cryopreserved samples. Follow the applicable procedure as needed.

4.2. **Fresh Peripheral Blood Isolation (Follow the instructions of the corresponding lymphocyte separation medium)**

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.

4.3. **Frozen Sample Processing**

Day -1 (Pre-treatment of Cryopreserved PBMCs): Cryopreserved PBMCs must be thawed and equilibrated 24 hours in advance. Thaw the cells in a 37°C water bath, then transfer to a biosafety cabinet. Transfer the cell suspension into a sterile centrifuge tube and slowly add 20 mL of **pre-warmed** NkVita Basal Medium dropwise while gently mixing. After the addition is complete, mix gently to ensure homogeneity. Centrifuge at 300×g for 5 minutes and discard the supernatant. Resuspend the cells in NkVita Immune Cell Serum-Free Medium (**prepared according to section 5.1**), and seed into culture flasks at a density of 2×10^6 cells/mL. Incubate overnight at 37°C.

V. NK Cell Expansion (Refer to Table 3)

5.1. **NK Expansion Medium** Preparation: Mix 1 L NkVita Basal Medium + 40 mL NkVita Supplement-02 + IL-2 (200 IU/mL).

5.2. Take PBMCs (for freshly isolated PBMCs, use 2×10^7 viable cells, or for cryopreserved PBMCs, after thawing and equilibrating for 24 hours as per step 4.3, count and take 2×10^7 viable cells). Thaw 1 vial of shownin® NK Expansion Reagent A, centrifuge to remove the supernatant (refer to step 4.3). Add 20 mL of the prepared medium from step 5.2 and mix thoroughly. After mixing, transfer the cell suspension into a T75 flask. Place the flask in a 37℃, 5% CO2 incubator and culture for 3 days.

5.3. **Day 0 – First Activation:** Use 2×10^7 viable PBMCs (fresh or 24 h post-thaw). Thaw 1 vial of shownin® NK Expansion Reagent A (follow 4.3), centrifuge, discard supernatant, resuspend in 20 mL prepared medium from 5.2, and transfer to a T75 flask. Incubate at 37°C, 5% CO2 for 3 days.

5.4. **Day 3 – Media Addition:** Add **10 mL** **NK Expansion Medium** to the flask and continue incubation.

5.5. **Day 4–7 – Daily Media Addition:** Based on medium color and cell density, add **NK Expansion Medium** to maintain a density of 1.0–1.5×10^6 cells/mL. When total volume exceeds 35 mL, transfer to a T175 flask.

5.6. **Day 7 – Second Activation (Optional):** Thaw shownin® NK Expansion Reagent B (see 4.3). Slowly add 27 mL pre-warmed serum-free medium. Resuspend in **NK Expansion Medium,** count, then add to culture vessel and adjust to 200 mL. Incubate at 37°C, 5% CO2 for 3 days.

*** The second activation on Day 7 is for reference only. Due to sample variability, there may be significant differences in cell expansion after the first activation. You need to observe and count the NK cells, and only proceed with the second activation if the total cell number has expanded 20 times (Day 7–9).**

5.7. **Day 8–13 –Media Addition:** Add **NK Expansion Medium** based on the color of the cell suspension or cell density. Ensure

that after each supplementation, the cell density remains between 1.0–1.5×10^6 cells/mL. Continue to calculate the volume of medium to add in the same way. When the total volume exceeds 200 mL, transfer to a cell culture bag for continued culture. After transferring to the culture bag, supplement every other day.

5.8. **Day 14–15 – Harvest Cells:** Optimal harvest time is typically Day 14 or 15.

Table 3: Table 3: Reference Volumes During Culture

Time	D0-D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12-14
Total Volume (mL)	20	30	50	100	200	400	1000	2000	2500	3000	4000
Operation	Add A	Media Addition	Media Addition	Media Addition Bottle Transfer	Medium Addition	Bag Transfer Add B Medium Addition	Medium Addition	Medium Addition	Medium Addition	Medium Addition	Medium Addition/ Counting and Harvesting
Container	T75			T175		Lymphocyte Culture Bag					

* This table is for reference only. Due to individual differences in samples, the volume of the culture medium may fluctuate. It is necessary to observe and analyze the growth condition of NK cells, and adjust the medium volume based on the optimal cell growth density.