ncKnight™ NK Expansion Factor Kit

Product Manual

Catalog # SN-03-0030 1 Kit

Product Introduction

ncKnight™ NK Expansion Factor Kit, is a GMP compliant, serum-free system specifically designed for the amplification of human natural killer (NK) cells. This system enables efficient expansion of **peripheral blood and cord blood**-derived NK cells, achieving a 5,000 to 10,000-fold increase in NK cell quantity within 14–15 days (calculated based on NK cells accounting for 10% of PBMCs). The protocol maintains high cell purity, with $CD3^-$ $CD56^+$ expression rates exceeding **80–95%**.

Product Information

Table 1. ncKnight™ NK Expansion Factor Kit Product Description

Product	Cat. No.	Amount	Storage
ncKnight™ NK Expansion Factor Kit Contains:	SN-03-0030*	1 Kit	-
ncKnight™ NK Medium Basal Medium	SN-03-0022	2 × 1000 mL	2–8 °C
ncKnight™ NK Medium Supplement	SN-03-0021	2 × 40 mL	-80 °C ~ -20 °C
ncKnight™ NK Expansion Factor A	SN-03-0031	1 × 270 µL	
ncKnight™ NK Expansion Factor B	SN-03-0032	1 × 120 µL	

* ncKnight™ NK Expansion Factor Kit is sold as a complete kit, components are not sold separately.

Recommended Reagent and Materials

Table 2. Recommended Reagents & Materials

Product	Cat. No.	Amount	Brand
ncKnight™ NK Expansion Factor Kit	SN-03-0030*	1 Kit	Shownin
NK-MAX Purification Factor	SN-03-0050	625 µL	Shownin
Lymphocyte Cryopreservation Medium	SN-06-1410	50 mL	Shownin
Ficoll-Paque PLUS	-	-	cytiva
Autologous Plasma / Human Platelet Lysate	-	-	-

Autologous Plasma Separation (Optional)

Centrifuge fresh blood at 900 × g for 20 minutes (with the elevation speed adjusted to the slowest), after centrifugation, aspirate the upper layer of yellowish plasma into a 50 mL tube (the remaining blood cell layer is used for isolating mononuclear cells), Place the tube in a 56 °C water bath for 30 minutes to inactivate the plasma. Then remove and centrifuge at 1200 × g for 10 minutes to remove the pellet. Transfer the inactivated plasma to a new 50 mL tube and store at 4 °C for future use.

Pretreatment blood with NK-MAX Purification Factor (Improve purity of expanded NK cells)

Add blood samples into a new tube with NK-MAX purification factor at a ratio of 12.5 µL/mL blood, mix gently and incubate at room temperature for 20 minutes, then isolate PBMCs with Ficoll-Paque PLUS.

Prepare peripheral blood mononuclear cells (PBMCs)

Isolate PBMCs from whole human blood by density gradient centrifugation using Ficoll-Paque PLUS. This PBMCs could use for NK expansion or cryopreserved with Lymphocyte Cryopreservation Medium (SN-06-1410, Shownin) for future use.

Prepare NK Complete Medium

Table 3. NK Complete Medium Mix ratio

Product	Cat. No.	Amount	Mix Ratio
ncKnight™ NK Medium Basal Medium	SN-03-0022	1000 mL	Mix 1000 mL Basal Medium and 40 mL Supplement with **200 IU/mL IL-2** as **NK Complete Medium.**
ncKnight™ NK Medium Supplement	SN-03-0021	40 mL	

NK cells Expansion from PBMCs

(2 L culture system), Suspend the PBMCs in **NK Complete Medium** at 1 × 10^6 cells/mL for totally 10 mL(total 1 × 10^7 PBMCs) supplemented with **5 %** (v/v) of autologous plasma or human platelet lysate in a T75 flask. Add **270 µL** of **ncKnight™ NK Expansion Supplement A** and **120 µL** of **ncKnight™ NK Expansion Supplement B** to the flask and incubate under 5 % CO_2 at 37 °C for 3 days.

(1 L culture system), Suspend the PBMCs in **NK Complete Medium** at 1 × 10^6 cells/mL for totally 5 mL(total 5 × 10^6 PBMCs) supplemented with **5 %** (v/v) of autologous plasma or human platelet lysate in a T25 flask. Add **135 µL** of **ncKnight™ NK Expansion Supplement A** and **60 µL** of **ncKnight™ NK Expansion Supplement B** to the flask and incubate under 5 % CO_2 at 37 °C for 3 days.

Day 3–7, add **NK Complete Medium** supplemented with **5 %** (v/v) of autologous plasma or human platelet lysate to ensure that the cell density as 1.0 × 10^6 cells/mL after.

Day 8–15 add **NK Complete Medium** supplemented with **1 %** (v/v) of autologous plasma or human platelet lysate to ensure that the cell density as 1.0 × 10^6 cells/mL after. The recommended liquid addition method can be referenced in the table below.

Harvesting NK cells, after culture for 14–16 days, the cell density should be at 2–3 × 10^6 cells/mL (totally **4–6 × 10^{10} NK cells for 2 L system**, **2–3 × 10^{10} NK cells for 1 L system**), then harvesting NK cells with centrifuge at 200 × g for 10 minutes.

Table4.Cultivation Scheme for 1 L and 2 L Systems

	Day	D0	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15
2 L culture system	Volume (mL)	10	10	15	20	35	70	150	300	450	600	1000	1600	2000	2000
	Vessel	T75 flask				T175/T225 flask			Culture bag						
1 L culture system	Volume (mL)	5	5	7	10	17	35	70	150	225	300	500	800	1000	1000
	Vessel	T25 flask			T175/T225 flask					Culture bag					