

ncKnight™ NK Expansion Factor Kit

Catalog # SN-03-0030 1 Kit (2 L)

Product Description

ncKnight™ NK Expansion Factor Kit, is a GMP compliant, serum-free culture 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 3,000 to 8,000-fold increase in NK cell quantity within 14 days. The protocol maintains high cell purity, with CD3- CD56+ cell expression rates exceeding 80-95%.

Product Information

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

Product	Cat. no./Part 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°C to 8°C	
ncKnight™ NK Medium Supplement	SN-03-0021	2 × 40 mL		
ncKnight™ NK Expansion Factor A	SN-03-0031	1 × 270 μL	-80°C to -20°C	
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.

Related Product

Table 2: Recommended Reagent & Material

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 min (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 12.5µL/mL blood, mix gently and incubate at room temperature for 20 minutes, then isolate PBMCs with FicoII-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
ncKnight™ NK Medium Supplement	SN-03-0021	40 mL	Complete Medium.

Culturing NK cells from PBMCs

(2 L culture system), Suspend the PBMCs in NK Complete Medium at 1 × 10⁶ cells/mL for totally 10 mL(total 1 × 10⁷ 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 % CO₂ 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 $\frac{5 \%}{6}$ (v/v) of autologous plasma or human platelet lysate in a T25 flask. Add $\frac{135 \ \mu L}{2}$ of ncKnightTM NK Expansion Supplement A and $\frac{60 \ \mu L}{2}$ of ncKnightTM NK Expansion Supplement B to the flask and incubate under 5 % CO₂ 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 $\frac{1 \%}{1 \%}$ (v/v) of autologous plasma or human platelet lysate to ensure that the cell density as 1.0 x 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 $2-3 \times 10^6$ cells/mL (totally 4-6 × 10^{10} NK cells for 2 L system, $2-3 \times 10^{10}$ NK cells for 1 L system), then harvesting NK cells with centrifuge at 200 ×g for 10 minutes.



	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			Cell 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			Cell culture bag							