

LymGro® NK 细胞体外培养试剂盒

Cat.No.	Product	Amount
CT011A	LymGro® NK 细胞体外培养试剂盒	Kit



Introductions

This kit is a pure cytokine-based, highly efficient NK cell in vitro culture kit without feeder cells. It is suitable for the expansion of NK cells from fresh and cryopreserved peripheral blood PBMCs and umbilical cord blood-derived UCBMCs. It features high expansion efficiency, high purity, high viability, and strong cytotoxicity. In a 2 L culture system, the expanded cell number can reach over 5 billion after 14–16 days of culture, and the purity of the sample can reach 90–99% after 14 days of culture. The operation is simple, saving culture space, and cell harvesting is easy, time-saving, and requires minimal consumables. A single expansion typically requires 20–30 mL of healthy human peripheral blood

Quality management system

The product is manufactured in CGMP standard workshop in Basalmedia Shanghai.

Basalmedia Shanghai Biotechnology Co.Ltd has obtained ISO9001 :2015 , ISO13485 :2016 quality system certification.

Kit Components

Product	Cat.No.	Amount	Number	Storage
NK Cell Culture Coating Solution	CT01A1	5mL	1	-20°C
NK Cell Activation Medium 1	CT01B1	20mL	1	-20°C
NK Cell Activation Medium 2	CT01C1	100mL	1	-20°C
ICGF (Immune Cell Growth Factor)	M250G V	100μL	1	-20°C
NK Cell Serum-free Medium	T521L0	1L	2	2~8°C
Cell Dilution and Washing Solution	B431K2	125mL	1	RT
Separation Tube	R715J8	50mL	2	RT

(containing 14 mL separation Liquid)				
1 L Bottom-Transparent Cell Culture Flask	LA-CF1000	1L	2	RT

Materials to be Prepared by the User

TC-treated T75 cell culture flasks, T150 cell culture flasks or larger-area culture flasks, pipettes, centrifuge tubes, etc

Operating Procedure

1. Coating:

- 1.1 Add 5 mL of NK Cell Culture Coating Solution (Cat. No.: CT01A1) to a T75 culture flask. Shake horizontally to mix and ensure the solution evenly covers the bottom surface. Incubate at 37 °C for 2 hours or at 4 °C overnight.
- 1.2 Before use, aspirate the coating solution. Add 10 mL of Cell Dilution and Washing Solution (Cat. No.: B431K2) to wash the bottom of the flask and repeat this wash step twice. The washed flask should be used immediately. During the pipetting and washing process, ensure that the pipette tip does not scratch the bottom of the flask.

2. Preparation of Autologous Plasma:

- 2.1 Add peripheral blood or umbilical cord blood into 50 mL conical centrifuge tubes, with a maximum volume of 30 mL per tube. Centrifuge at $700 \times g$ for 15 min (acceleration set to 6, deceleration set to 1).
- 2.2 Aspirate the upper pale yellow plasma layer and transfer to a new 50 mL centrifuge tube. Inactivate in a 56 °C water bath for 30 min. The remaining portion in the centrifuge tube will be used for the isolation of mononuclear cells.
- 2.3 Centrifuge the inactivated plasma at $900 \times g$ for 10 min (acceleration set to 9, deceleration set to 9). Collect the supernatant and store at 4 °C for subsequent use, or store at -80 °C for long-term storage.

3. Preparation of Mononuclear Cells

- 3.1 Take the lower cell layer obtained after centrifugation in

step 2.2, dilute 1:1 with Cell Dilution and Washing Solution, and mix well. Slowly layer 21–25 mL of the diluted blood onto each 50 mL Separation Tube pre-loaded with lymphocyte separation medium (Cat. No.: R715J8), ensuring a clear interface between the two layers. Centrifuge at $400 \times g$ for 10 min at room temperature (acceleration set to 6, deceleration set to 1).

3.2 Transfer the mononuclear cell layer (buffy coat) to a new 50 mL centrifuge tube. Add Cell Dilution and Washing Solution to bring the volume to 50 mL. Centrifuge at $380 \times g$ for 10 min (acceleration set to 6, deceleration set to 5).

3.3 Discard the supernatant. Resuspend the cells in Cell Dilution and Washing Solution and bring the volume to 40 mL. Take a sample for counting. Centrifuge the remaining cell suspension at $380 \times g$ for 10 min (acceleration set to 6, deceleration set to 5).

4. Preparation of Expansion Medium:

Expansion Medium: Add 50 μ L of ICGF (Immune Cell Growth Factor, Cat. No.: M250GV) to each 1 L bottle of NK Cell Serum-Free Medium (Cat. No.: T521L0). Mix well and set aside. Store at 2–8 °C; it is recommended to use within one month.

5. NK Cell Induction, Activation, and Proliferation

Day -1: Coat the T75 culture flask.

Day 0: Based on the total cell number obtained in step 3.3, add 18 mL of NK Cell Activation Medium 1 (Cat. No.: CT01B1) to resuspend the mononuclear cells and adjust the density to $1.0\text{--}1.5 \times 10^6$ cells/mL. Add the cell suspension to the coated T75 flask. Supplement with 2 mL of autologous plasma. Culture in a 37 °C, 5.0% CO₂ incubator. During culture, avoid moving the flask as much as possible; handle gently when observing.

Day 3: Add 38 mL of NK Cell Activation Medium 2 (Cat. No.: CT01C1) and supplement with 2 mL of autologous plasma, bringing the total volume to 64 mL. During culture, avoid moving the flask as much as possible; handle gently when observing.

Day 5: Transfer the culture to a T150 flask. Add 57 mL of Activation Medium 2 and supplement with 3 mL of autologous plasma, bringing the total volume to 120 mL.

Day 7: Transfer into a 1 L Bottom-Transparent Flask (Cat. No.: LA-CF1000). Add 116 mL of the expansion medium prepared in step 4 and supplement with 4 mL of autologous plasma, bringing the total volume to 240 mL.

Day 8: Equally split the culture into two 1 L Bottom-Transparent Flasks. At the same time, add 120 mL of expansion medium to each flask. Divide the remaining autologous plasma equally between the two flasks, bringing the total volume in each flask to 240 mL.

Day 10: Aspirate and discard 100 mL of the supernatant medium from the bottom-transparent flasks, being careful not to aspirate the settled cells at the bottom. Add 340 mL of expansion medium to each flask, bringing the total volume in each flask to approximately 480 mL.

Day 12: Aspirate and discard 200 mL of the supernatant medium from the bottom-transparent flasks, being careful not to aspirate the settled cells. Add 480 mL of expansion medium to each flask, bringing the total volume in each flask to approximately 760 mL. Take samples for quality control and flow cytometry analysis.

Day 14: Take samples for cell counting and harvest the NK cells (total volume approximately 1520 mL). Take samples for quality control and flow cytometry analysis.

Related Products

Cat.No.	Product	Amount	Storage	Transportation
T110KJ	LymGro™ Serum-free Lymphocyte Culture Medium containing HSA	500 mL	2 ~ 8 °C	Blue Ice
T120KJ	LymGro™ Serum-free Lymphocyte Culture Medium, Animal Component and Phenol red-free	500 mL	2 ~ 8 °C	Blue Ice
T150L0	Sample Density Separation Medium, 1.077	1 L	2 ~ 8 °C	Blue Ice

T520L0	L-Glutamine Solution, 100X *	1 L	2 ~ 8 °C	Blue Ice
T521L0	L-Alanyl-L-Glutamine Solution, 100X *	1 L	2 ~ 8 °C	Blue Ice
R710JV	Dulbecco's DPBS, without Ca ²⁺ , Mg ²⁺ , Phenol red	100 mL	2 ~ 30 °C	RT
S110JV	Phosphate-Buffered Saline (PBS), pH7.2	100 mL	-30 ~ -5 °C	Dry Ice
S210JV	Phosphate-Buffered Saline (PBS), pH7.4	100 mL	-30 ~ -5 °C	Dry Ice
S240JV	LymGro™ Serum-free Lymphocyte Culture Medium containing HSA	100 mL	2 ~ 8 °C	Blue Ice
B210KJ	LymGro™ Serum-free Lymphocyte Culture Medium, Animal Component and Phenol red-free	100 mL	2 ~ 30 °C	RT
B310KJ	Sample Density Separation Medium, 1.077	500 mL	2 ~ 30 °C	RT
B320KJ	L-Glutamine Solution, 100X *	500 mL	2 ~ 30 °C	RT

* 100X indicates that the product concentration is 100 times the working concentration.