

CelThera™ NK Cell Expansion Medium (Phenol Red-free) User guide

Cat. No.: NK-CM31

Product Overview

NK cell expansion medium is a serum-free, animal-derived, and antibiotic-free medium, which can maintain NK cell expansion. Compared with the medium containing animal serum, the serum-free medium greatly reduces the risk of introducing heterologous substances during the culture of NK cells. At the same time, the components of the medium are more stable which can improve the batch consistency of the medium. The serum-free medium for NK cell expansion is suitable for the cytokines activation system and feeder cell activation system, and it could be used with heat-inactivated autologous plasma or human AB serum.

Product Specifications

Cat. No	Contents	Amount	Storage	Shelf life
CM3102	CelThera™ Immune Cell Basal Medium (Phenol Red-free)	1000ml	2°C -8°C Protect from light.	18 months
CM31S2	CelThera™ Immune Cell Supplement C	8ml	-20°C or below. Protect from light.	12 months

Method of Use

Preparation complete medium:

Thaw CelThera™ Immune Cell Supplement C (Cat. No. CM31S2) to room temperature. In a biosafety cabinet, remove the lid on both CelThera™ Immune Cell Supplement C and CelThera™ Immune Cell Basal Medium (Phenol Red-free). Add 8mL of supplement C into 1L of basal medium and close the lid. Mix upside down 3 to 5 times and store the mixed complete culture medium at 2°C to 8°C. It is recommended that the mixed complete culture medium be used within 4 weeks.

**Please note: this complete medium does NOT contain cytokines. For NK cell expansion, additional cytokines such as IL-2, or IL-15 and so on, are needed. The amount of cytokines or other growth factors needed will vary depending on your specific NK cell application.*

Activation and expansion of NK cells in PBMC:

Fresh PBMC, antibody or cytokines activation system, and addition of AB serum were used as examples

1. Pretreatment of culture flask: Take the cell culture well plate or culture flask, add the coating factor, shake it from side to side, so that the liquid is evenly dispersed in the bottom of the flask or the bottom of the well,

seal the flask with the sealing membrane, and put it in 2-8°C overnight for activation.

2. On day 0, the culture flask or plate activated overnight at 2-8°C was removed, and the coating solution was discarded. The PBMC were resuspended in NK medium (10% AB serum) and inoculated in a culture plate or bottle (recommended density of PBMC: 1.5×10^6 cells/mL), supplemented with an appropriate amount of activating antibody and cytokines, and cultured in 37°C, 5% CO₂ incubator.

3. On day 3, the same amount of fresh NK medium (10% AB serum) and an appropriate amount of cytokines were slowly added along the side wall of the culture well or culture flask as on day 0. Note: Do not touch the bottom of the culture flask or culture well, do not blow cells, count, reduce observation and other operations to avoid affecting the initial growth of cells.

4. On the 5th day, fresh NK medium (adding 5% AB serum) and an appropriate amount of cytokines were added, the cell density was adjusted to $3-3.5 \times 10^5$ cells/mL, and the well or flask was expanded according to the volume of cell suspension.

5. On the 7th day, fresh NK medium (1-2% AB serum) and an appropriate amount of cytokines were added, the cell density was adjusted to $2.5-3 \times 10^5$ cells/mL, and the well or flask was expanded according to the volume of cell suspension.

6. From the 7th day, fresh NK medium (1-2% AB serum) and an appropriate amount of cytokines can be added every 3 days, the cell density can be adjusted to $2.5-3 \times 10^5$ cells/mL, and the well or flask can be expanded according to the volume of cell suspension.

7. Cells were harvested at 12-14 days of culture.

Note:

The medium was equilibrated to room temperature for use.

The medium could be used for both PBMC and CBMC.

For CBMC, on day 0, the recommended inoculation density of CBMC was 2.5×10^6 cells/mL, and the passage procedure and recommended passage density were the same as PBMC.

Related Products

- GMP Human IL-2 Protein (Cat. No. GMP-L02H14)
- GMP Human IL-12 Protein (Cat. No. GMP-L12H23)
- GMP Human IL-15 Protein (Cat. No. GMP-L15H13)
- GMP Human IL-18 Protein (Cat. No. GMP-L18H16)
- GMP Human IL-21 Protein (Cat. No. GMP-L21H25)
- GMP Monoclonal Anti-Human CD3 Antibody (OKT3) (Cat. No. GMP-MC0323)
- GMP Human 4-1BB Ligand Protein, Fc Tag (Cat. No. GMP-41LH26)