

CelThera™ NK Cell Activation and Expansion Kit (Phenol Red-free)

Cat. No. NK-CM32

Product Overview

This kit is designed for culturing natural killer (NK) cells, utilizing a pure cytokine activation system. It is formulated to be serum-free, free of animal-derived components, and antibiotic-free. Compared with kits containing animal-derived components or serum, it significantly reduces the risk of introducing heterologous substances during NK cell culture. Additionally, the absence of serum and animal-derived components ensures better batch-to-batch consistency of the kit. The CelThera™ NK Cell Activation and Expansion Kit (Phenol Red-free) requires supplementation with human AB serum or autologous plasma for use.

Component List

The kit offers 1-3L medium systems, suitable for samples derived from 10-30ml peripheral blood or cord blood. The components with different catalog numbers, their specifications, storage conditions, and shelf lives are as follows:

Cat. No.	Component Name	1L Specification	2L Specification	3L Specification	Storage	Shelf Life
CM3102	CelThera™ Immune Cell Basal Medium (Phenol Red-free)	1000ml*1	1000ml*2	1000ml*3	2°C-8°C, protect from light	18 months
CM31S2	CelThera™ Immune Cell Supplement C	8ml*1	8ml*2	8ml*3	-20°C or below, protect from light	12 months
CM31S4	CelThera™ NK Cell Expander 1	240ul*1	240ul*1	240ul*1	-20°C or below, protect from light	24 months
CM31S5	CelThera™ NK Cell Expander 2	240ul*1	240ul*1	240ul*1	-20°C or below, protect from light	24 months
CM31S6	CelThera™ NK Cell Expander 3	0.12ug*1	0.12ug*1	0.12ug*1	-20°C or below, protect from light	24 months
CM31S7	CelThera™ NK Cell Expander 4	200ul*1	200ul*1	200ul*1	-20°C or below, protect from light	24 months
CM31S8	CelThera™ NK Cell Expander 5	1ml*1	1ml*2	1ml*3	-20°C or below, protect from light	24 months

Note:

- Components stored at -20°C or below should be thawed at room temperature or 4°C.
- A complimentary bottle of CelThera™ GMP Phenol Red Solution (0.5%) (Cat# GMP-PI1100) is supplied with this kit. For cell culture procedures requiring phenol red indicator, add 1.5 mL of this solution to 1 L of CelThera™ Immune Cell Basal Medium (Phenol Red-free) and mix thoroughly.

Usage Instructions

A. Medium Preparation

1. Complete Medium:



Add 8ml of thawed Supplement CM31S2 to 1000ml of Basal Medium CM3102, mix well, and store at 2-8°C for use within 3-4 weeks.

2. Coating Diluent:

Add the entire volume of thawed Expander 1 to 24ml of DPBS. Rinse the Expander 1 vial with 1-2ml of DPBS and add the rinse to the DPBS, then mix well.

3. Activation Medium:

Dissolve the lyophilized Expander 3 in the entire volume of thawed Expander 4, mix thoroughly, and add to 150ml of Complete Medium. Rinse the Expander 4 and Expander 3 vials with 1-2ml of Complete Medium, add the rinse to the Complete Medium, and mix well. Tips: Store the Activation Medium at 2-8°C for use within 1 week.

3.1 Initial Activation Medium (Day 0-Day 3):

Add the entire volume of thawed Expander 2 to 24ml of Activation Medium. Rinse the Expander 2 vial with 1-2ml of Activation Medium, add the rinse to the Activation Medium, and supplement with 10% human AB serum or autologous plasma. Tips: Prepare immediately before use.

3.2 Activation Medium (Day 3-Day 5):

Take 24ml of Activation Medium and supplement with 10% human AB serum or autologous plasma. Tips: Prepare immediately before use.

3.3 Activation Medium (Day 5-Day 7):

Take the remaining 100ml of Activation Medium and supplement with 5% human AB serum or autologous plasma. Tips: Prepare immediately before use.

4. Expansion Medium:

Add 1ml of thawed Expander 5 to 1000ml of Complete Medium (if part of the Complete Medium has been used in previous steps, calculate the volume of Expander 5 required based on a 1:1000 volume ratio of Expander 5 to remaining Complete Medium, and add accordingly). Supplement with 1% human AB serum or autologous plasma, mix well, and store at 2-8°C for use within 1-2 weeks.

B. NK Cell Activation and Expansion Culture (Taking PBMC with AB Serum as an Example)

1) Day -1:

Add the Coating Diluent to a T75 culture flask, ensuring uniform distribution on the flask bottom. Seal the flask with parafilm and incubate at 2-8°C for overnight coating.

2) Day 0:

Remove the T75 flask from 2-8°C and equilibrate to room temperature.

Resuspend PBMC in 20ml of Initial Activation Medium (Day 0-Day 3) at a recommended seeding density of 1.5×10^6 cells/ml.

Aspirate and discard the coating solution from the T75 flask.

Add the resuspended PBMC to the T75 flask, ensure uniform cell distribution, and incubate in a 37°C CO₂ incubator.

3) Day 3:

Gently add 20ml of Activation Medium (Day 3-Day 5) along the sidewall of the T75 flask to avoid disturbing the cells at the bottom. Do not pipette or count cells at this stage to prevent affecting early-stage growth. Continue incubation in a 37°C CO₂ incubator.

4) Day 5:

Remove the T75 flask from the incubator, take a sample for cell counting, supplement with Activation Medium (Day 5-Day 7), and scale up the culture into additional flasks based on the total cell count:

If total cell count $< 4 \times 10^7$: Supplement with 40ml of Activation Medium (Day 5-Day 7) and seed into 2 T75 flasks.

If total cell count $\geq 4 \times 10^7$: Supplement with 80ml of Activation Medium (Day 5-Day 7) and seed into 3 T75 flasks. Continue incubation.

5) Day 7:

Discounts, Gifts,
and more!



» » www.acrobiosystems.com

Remove the flask/bag from the incubator, take a sample for cell counting, adjust the cell density to $3-4 \times 10^5$ cells/ml with Expansion Medium, transfer to culture flasks or bags, and continue incubation.

6) Day 9:

Repeat the cell counting, adjust the density to $3-4 \times 10^5$ cells/ml with Expansion Medium, scale up the culture, and continue incubation.

7) Day 11:

Repeat the cell counting, adjust the density to $3-4 \times 10^5$ cells/ml with Expansion Medium, scale up the culture, and continue incubation.

8) Day 13-15:

Remove the flask/bag from the incubator, take a sample for cell counting, harvest cells by centrifugation.

Important Notes:

- A) Equilibrate the medium to room temperature before use.
- B) Avoid repeated freeze-thaw cycles for Expanders 1, 2, 3, 4, and 5.
- C) The kit is suitable for both PBMC and CBMC.
- D) The recommended initial seeding density for CBMC is 2.5×10^6 cells/ml; subculture procedures and recommended densities are the same as for PBMC.
- E) If the initial PBMC or CBMC quantity is low, adjust the volumes according to the following table:

Culture Vessel	Coating Diluent	Initial Activation Medium (Day 0-Day 3)	PBMC Seeding Density	CBMC Seeding Density
T25 Flask	8ml	8ml	1.5e6 cells/ml	2.5e6 cells/ml
6-Well Plate (per well)	3ml	3ml	1.5e6 cells/ml	2.5e6 cells/ml
12-Well Plate (per well)	1ml	1ml	1.5e6 cells/ml	2.5e6 cells/ml