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

Cat. No. GMP-NKCM37

Product Overview

This kit is designed for culturing natural killer (NK) cells, utilizing a pure cytokine activation system. It is serum-free, animal-derived free, and no antibiotics are added during manufacturing. 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™ GMP NK Cell Activation and Expansion Kit (Phenol Red-free) requires supplementation with human AB serum or autologous plasma for use.

Component List

Cat. No.	Component Name	5L Specification	Storage	Shelf Life
GMP-CM3102A	CelThera™ GMP Immune Cell Expansion Medium (Phenol Red-free)	1000ml*5	2℃-8℃, protect from light	18 months
GMP-CM31S2	CelThera™ GMP Immune Cell Supplement C	8ml*5	-20℃ or below, protect from light	24 months
GMP-CM31S4	CelThera™ GMP NK Cell Expander 1	240ul*1	-20℃ or below, protect from light	24 months
GMP-CM31S5	CelThera™ GMP NK Cell Expander 2	240ul*1	-20℃ or below, protect from light	24 months
GMP-CM31S6	CelThera™ GMP NK Cell Expander 3	200ul (after reconsititution)*1	-20℃ or below, protect from light	24 months
GMP-CM31S7	CelThera™ GMP NK Cell Expander 4	200ul*1	-20℃ or below, protect from light	24 months
GMP-CM31S8	CelThera™ GMP NK Cell Expander 5	1ml*5	-20℃ or below, protect from light	24 months

Note:

1. Components stored at -20℃ or below should be thawed at room temperature or 4℃.
2. Five complimentary bottles 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™ GMP Immune Cell Expansion Medium (Phenol Red-free) and mix thoroughly.
3. To ensure consistent performance, Expander 3 (GMP-CM31S6) is supplied as lyophilized powder and is recommended to be used immediately after reconsitution with Expander 4 (GMP-CM31S7).

Usage Instructions

A. Medium Preparation

1. Complete Medium:

Add 8ml of thawed Supplement GMP-CM31S2 to 1000ml of Basal Medium GMP-CM3102A, mix well, and store at 2-8℃ 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℃ 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℃ 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℃ for overnight coating.

2) Day 0:

Remove the T75 flask from 2-8℃ 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℃ CO_2 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℃ CO_2 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 cell density <1e6 cells/ml: Supplement with 40ml of Activation Medium (Day 5-Day 7) and seed into 2 T75 flasks.

If cell density ≥1e6 cells/ml: Supplement with 80ml of Activation Medium (Day 5-Day 7) and seed into 3 T75 flasks or one T175 flask.

If cell density ≥2e6 cells/ml: Supplement with 100ml of Activation Medium (Day 5-Day 7) and seed into 2 T175 flasks or one T225 flask.

Continue incubation.

5) Day 7:

Remove the flask/bag from the incubator, take a sample for cell counting, adjust the cell density to $3-4\times10^5$ 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 3-4×10^5 cells/ml with Expansion Medium, scale up the culture, and continue incubation.

7）Day 11:

Repeat the cell counting, adjust the density to 3-4×10^5 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 Expander 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; For the first 7 days, follow the same procedures as for PBMC.

From day 7, adjusting the cell density to 5-6e5/ml with Expansion Medium every two days is recommended.

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 (Day0-3)	PBMC Seeding Density	CBMC Seeding Density	Activation Medium (Day3-5)	Activation Medium (Day5-7)
T25 Flask	8ml	6ml	1.5e6 cells/ml	2.5e6 cells/ml	6ml	If cell density <1e6 cells/ml, supplement with 12ml; If cell density ⩾1e6 cells/ml, supplement with 24ml If cell density ⩾2e6 cells/ml, supplement with 30ml
6-Well Plate (per well)	3ml	2.5ml	1.5e6 cells/ml	2.5e6 cells/ml	2.5ml	If cell density <1e6 cells/ml, supplement with 5ml; If cell density ⩾1e6 cells/ml, supplement with 10ml If cell density ⩾2e6 cells/ml, supplement with 12.5ml
12-Well Plate (per well)	1.2ml	1ml	1.5e6 cells/ml	2.5e6 cells/ml	1ml	If cell density <1e6 cells/ml, supplement with 2ml; If cell density ⩾1e6 cells/ml, supplement with 4ml If cell density ⩾1e6 cells/ml, supplement with 5ml