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Human NK Cell Expansion Kit

Product Number: CK001

1. Introduction

This document describes the recommended usage instructions for the Human NK Cell Expansion Kit (Catalog Number K001) for NK cell expansion. The kit contains three lyophilized products: K001-1A, K001-2A, and K001-3A. This kit is designed to be used with an initial culture medium. The specific usage recommendations are as follows:

2. Product Reconstitution

CK001-1A: Dissolve the lyophilized powder in 100 μL of water, then add 7.4 mL of PBS to prepare a coating solution; total volume is 7.5 mL. Store at -20°C or below.

CK001-2A: Dissolve the lyophilized powder in 100 μL of water, then add 9.9 mL of initial culture medium to prepare an activation factor; total volume is 10 mL. Store at -20°C or below.

CK001-3A: Dissolve the lyophilized powder in 270 μL of water, then add 9.73 mL of initial culture medium to prepare an expansion factor; total volume is 10 mL. Store at -20°C or below.

3. Usage Summary Table

Step	Medium
Coating	CK001-1A, 7.5 mL coating solution
Activation Medium	190 mL initial culture medium + 10 mL CK001-2A: Mix the two together for use
Expansion Medium	1800 mL initial culture medium + 10 mL CK001-3A: Mix the two together for use

4. Brief Operation Process

4.1 Day 0, Coating: Add 7.5 mL of CK001-1A coating solution to a T75 culture flask and incubate at 37°C for more than 3 hours.

4.2 Add PBMCs isolated from whole blood or thawed PBMCs to the pre-coated T75 cell culture flask. Based on the counting results, supplement with activation culture medium and control the cell density to 1.5-2 x 10^6/mL (the culture temperature can be set to 38.5°C for 4 hours during activation). After 4 hours of culture at 38.5°C, transfer to a 37°C, 5% CO_2 incubator overnight.

4.3 Day 1: Pre-warm the activation medium at 37°C. Remove the NK cells from the incubator and add 1-2 times the volume of pre-warmed activation medium based on the cell growth status. Culture in a 37°C, 5% CO_2 incubator for 48 hours.

4.4 Day 3: Pre-warm the activation medium at 37°C. Remove the NK cells from the incubator, transfer the cell suspension to a 50 mL centrifuge tube, and gently rinse the bottom of the culture flask with fresh NK culture medium (initial culture medium) to collect adherent cells as much as possible. Centrifuge at 400g for 5 minutes, remove the supernatant, resuspend in 5 mL of fresh NK culture medium (initial culture medium), mix well, and count the cells. Based on the counting results, seed at a density of 1.2-1.5 x 10^6/mL into a new T75 culture flask. Culture in a 37°C, 5% CO_2 incubator for 48 hours.

4.5 Day 5 (equivalent to Day 0 of NK cell expansion): Pre-warm the expansion medium at 37°C. Remove the NK cells from the incubator, observe under a microscope, and count the cells. After centrifugation, add expansion culture medium at a density of 0.8-1 x 10^6/mL. Culture in a 37°C, 5% CO_2 incubator for 48 hours, then add 1-2 times the volume of expansion culture medium every other day.

4.6 Optimize the cell harvest time based on specific needs; the maximum culture period is 21 days.

5. Precautions

5.1 CK001-1A, CK001-2A, and CK001-3A should be reconstituted just before use. If not formulated into culture medium after reconstitution, store at -20°C or below to avoid repeated freeze-thaw cycles. The validity period is 6 months.

5.2 Once the kit is mixed with the initial culture medium to prepare the activation or expansion medium, it should be used within one

month when stored at 2-8°C.