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Instructions for Use of NK cell Expansion Kit

Cat.No. HG-POC004

The Reagent is intended for research use only, not for diagnostic purposes.

CONTENTS

1. Product Description

NK cell expansion kit consists of trophoblast cells that stimulate and activate NK cells (NK/TIL Cell Expansion Reagent) and NK cell basal medium.NK/TIL Cell Expansion Reagent is irradiated and inactivated engineered K562 cells that express various cytokines such as IL-21, and paired with the NK cell basal medium, NK cells from umbilical cord blood and peripheral blood single nucleus cell sources are directed to activate and expand in large numbers with high purity of the obtained NK cells. Under the synergistic signaling effect of various cytokines, NK cells derived from umbilical cord blood and peripheral blood single nucleated cells can be activated and expanded in a targeted manner, and the NK cells obtained are of high purity. This product is for in vitro research only.

2. Production Composition

Product Content	Cat No	Composition	Specification	Amount	Preservation condition
NK cell Expansion Kit	HG-POC004	NK/TIL cell expansion Kit	1E7/1mL	1	Liquid Nitrogen
			4E7/4mL	1	
		NK cell basal medium	1000ml	1	2~8°C

3. Usage and Operation Steps

3.1 Reagent and Consumables

Serial number	Name		Dosage (at ~1-2E6 PBMC initiation)
1	Reagent	NK/TIL cell expansion Kit	1mL×1+4mL×1
2		NK cell basal medium	1L
3		IL-2	4 vial，200 thousands IU/vial
4		0.9%NaCl	500mL
5		20% human albumin	15mL
6		Ficoll	25mL
7	Consumables	Cell count plate	Several
8		6 Well palte	1
9		T25 Flask	1
10		T75 Flask	2
11		T175 Flask	3
12		640cm^2 cell culturebag	1
13		15mL centrifuge tubes	Several
14		1.5mL centrifuge tubes	Several
15		10mL, 25mL pipettes	Several
16		1 ml and 200 µL pipette tips	Several

3.2 Key points and steps of NK cell expansion and culture

3.2.1 NK cell complete medium preparation: NK cell basal medium + autologous plasma (D0-D8: 3%; D8-D10:1%; D10 without add) + IL-2 (400 IU/mL)

3.2.2 D0 plasma and PBMC isolation and inoculation

3.2.2.1 Autologous plasma separation: Fresh blood is centrifuged at 800g for 15min, the upper layer of pale yellow plasma is sucked into a 50mL centrifuge tube, and inactivated in a water bath at 56°C for 30min, then 800g is centrifuged for 10min to remove the precipitate, the upper plasma is transferred to a new 50mL centrifuge tube, sodium heparin (final concentration is 40IU/ml), mixed well and stored in a 4° C refrigerator for later use. The lower layer of blood cells is used to extract mononuclear PBMCs.

3.2.2.2 Separation of PBMCs: the same volume of normal saline was mixed with the blood cell pellet, slowly added to the Ficoll layer (diluted blood cells: Ficoll=2:1) to keep the stratification clear, centrifugation at room temperature at 400g for 30min, increase speed 4, speed decrease 3. Then carefully suck the middle buffy film layer, add normal saline to blow and mix well, centrifuge at 400g at room temperature for 10min, discard the supernatant, and repeat the washing of PBMC again, discard the supernatant, resuspend the cells with an appropriate amount of complete medium and take samples for counting.

3.2.2.3 Recovery of NK/TIL cell expansion reagent (1E7/piece): 37°C water bath to recover NK/TIL cell expansion reagent (1E7/piece), and after recovery, NK/TIL cell expansion reagent (1E7/piece) was put in an appropriate amount of thawing solution (0.9% NaCl injection + 5% HSA). Centrifuge and wash once at 400g and 10min, discard the supernatant and resuspend it with an appropriate amount of NK cell complete medium, and wash it again with 400g and 10min centrifugation, discard the supernatant, and add 1ml of NK cell complete medium to resuspend evenly.

3.2.2.4 Inoculation: According to the results of PBMC, 1E7 PBMC was added to the resuspended NK/TIL cell expansion reagent (1E7/piece), the mixed PBMC and NK/TIL cell expansion reagent (trophoblasts) were transferred to a suitable culture container, and NK cell complete medium was added to 40ml to adjust the PBMC density to 2.5E5/ml, and cultured at 37°C and 5% CO_2 conditions.

3.2.3 D4 Observe the fluid exchange

Observe the cells, mix well, transfer to a centrifuge tube, centrifuge at 400g for 10min, and the cell pellet is resuspended with 80ml of NK cell complete medium and transferred to a T175 bottle, where it is cultured at 37°C and 5% CO_2 .

3.2.4 D6 Observe rehydration

Observe the cells, mix well, take samples and count, add NK cell complete medium and adjust the cell density to 5E5/ml according to the color of the cell suspension and the cell status under the microscope, and inoculate into one or more T175 bottles according to the total volume, each T175 bottle volume should not exceed 120ml, and culture at 37°C, 5% CO_2 conditions.

3.2.5 D8 observation, secondary activation, and rehydration

Observe the cells, mix well, and take samples to count. Then, NK/TIL cell expansion reagent (4E7) was recovered, washed and sampled and counted (see 3.2.2.3), 4E7 NK cells were mixed with the resuspended NK/TIL cell expansion reagent (4E7), NK cell complete medium was added to 160ml to adjust NK cell density to 2.5E5/ml, seeded in two T175 culture flasks, each bottle was 80ml, and cultured at 37°C, 5% CO_2 conditions.

3.2.6 D10 Observe the fluid exchange

Observe the cells, mix well, take samples and count, centrifuge the remaining cells at 400g for 10min, adjust the cell density to 5~8E5/ml after resuspension with NK cell complete medium, and transfer to a 640cm^2 cell culture bag for culture.

3.2.7 D12 Observe rehydration

Observe the cells, mix well, take samples and count, and add all the remaining medium.

3.2.8 D14 harvest

Observe the cells, sample and count, and send NK cells for purity according to the cell count results, and the remaining cells can be harvested and cryopreserved.

Note:This NK cell culture step is for reference only, due to individual differences in samples, adjustment of culture methods and other factors, there may be differences in the expansion and culture status of NK cells, at this time, appropriate adjustments can be made after observation and analysis of NK cell growth.

4. Note

4.1 Blood collection: It is recommended to use a heparin sodium anticoagulant tube or a sodium citrate blood collection bag.

4.2 Autologous plasma will affect the culture status of cells, and it is recommended that the autologous plasma reserve is about 30mL. Hemolysis and high fat may affect the expansion status of NK cells.

4.3 This reagent is only used for in vitro induction culture for the purpose of obtaining NK cells, and the components of the reagent from different batches cannot be used interchangeably.

4.4 If there is a crack in the outer packaging tube of the reagent, it should be discontinued immediately.

4.5 The operation process should be carried out in a sterile environment, and all reagent consumables used in the operation process that are in direct contact with the cell fluid must be strictly sterile.

4.6 This reagent must be used at one time after opening, and should not be used after repeated freezing and thawing.

4.7 In order to ensure the activity of this reagent, the recovery should be carried out in strict accordance with the recovery requirements of NK and TIL cell expansion reagents. It is strictly forbidden to directly submerge the cryovial tube containing the amplification reagent in the water bath during cell recovery, and it is recommended to at least ensure that the tube cap is above the water bath surface.

4.8 It is normal for cells to form clumps in the early stage of NK cell culture, and please be careful not to destroy the cell clumps during the operation, so as not to affect the later expansion of NK cells.

4.9 Use of culture medium: The medium needs to be naturally rewarmed at room temperature before each rehydration, and the complete medium is ready to use.

4.10 The washing liquid and various wastes generated during the experiment should be disposed of in accordance with the corresponding regulations and the requirements of local regulatory authorities.

5. Storage Conditions

5.1 liquid nitrogen.

6. Expiry Date:

6.1 36 months.

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