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Instructions for Use of NK and TIL Cell Expansion Reagents (K562 Feeder Cell)

Cat.No. HG-FEC001-RG

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

CONTENTS

1. Product Description

At present, NK and TILs and other related immune cell products have great therapeutic potential for tumor cells (especially solid tumors), and are expected to be developed into universal cell products to meet the needs of different patients due to their low immunogenicity, while large-scale cell expansion is a common bottleneck faced by NK and TILs and other related immune cells, and studies have shown that trophoblast cells treated with high-energy irradiation can lose the ability to divide and proliferate without affecting cell metabolism. Research has shown that trophoblast cells treated with high-energy irradiation can lose the ability to divide and proliferate without affecting cell metabolism, but the cells are intact, which can promote the activation and expansion and toxicity of certain immune cells with limited expansion in vitro, and will eventually be completely killed by the immune cells and cleaved into cellular debris, which can be removed from the final product through washing. Currently, several NK cells and TILs cell products produced using trophoblast cells have been approved for clinical use or marketed globally, and studies have shown that genetically modified K562 trophoblast cells can achieve more than 10,000-fold expansion of NK or CAR-NK cells in 15 days, which can meet the demand for their large-volume preparation. Therefore, the development of off-the-shelf trophoblast cell products that can promote the proliferation of immune cells such as NK and TILs and act as antigen presenters is of great significance in meeting the demand for large-scale expansion of cell therapeutic products (e.g., CAR-NK/NK, TILs, etc.) at different stages of drug development in the global market.

Hillgene's NK/TIL Cell Expansion Reagent (Feeder cell) is an irradiated and inactivated engineered K562 cells expressing IL-21 and other cytokines, which, together with the mainstream immune cell culture medium, can activate and expand NK cells and tumor-infiltrating TIL cells derived from umbilical cord blood and peripheral blood mononuclear cells cultured in vitro, and the purity of the obtained NK and TIL cells is high.

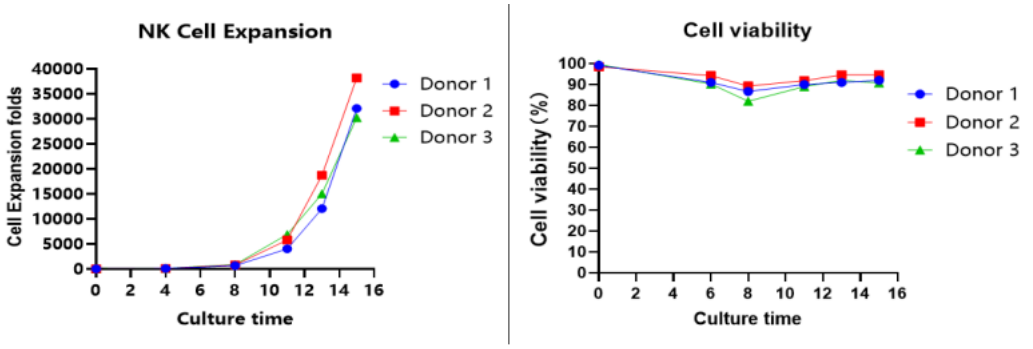

Figure 1. Fold expansion and viability of different Donor-derived NK cells

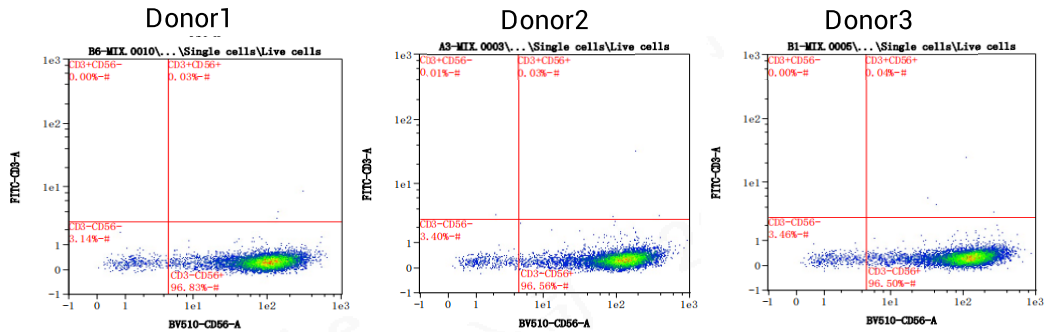

Figure 2. Purity of different Donor-derived NK cells

2. Product components

2.1 NK/TIL Cell Expansion Reagent products should be stored in liquid nitrogen and have a product expiration date of three years.

2.2 If you have other specifications, please contact us for customized production.

3. Laboratory materials prepared by the laboratory itself (not provided)

Serial number	Name of reagent and consumables	Dosage (at ~1-2E6 PBMC initiation)
1	serum-free medium	2L
2	IL-2	4 sticks, 200,000 IU/stick
3	0.9% NaCl injection	Approx. 500 mL
4	20% human serum albumin	Approx. 15 mL
5	Lymphocyte isolation solution Ficoll	Approx. 25 mL
6	NC-200 counting board (or other counts)	a certain number or amount
7	6-well plate	1 个
8	T25 flask	1
9	T75 flask	1
10	T175 flask	2
11	640cm^2 cell culture bag	2
12	15mL centrifuge tube	1
13	1.5mL centrifuge tube	a certain number or amount
14	10mL、25mL pipette	a certain number or amount
15	1mL and 200μL pipette tips	a certain number or amount

4. General considerations and biosafety guidelines

- Please follow Biosafety Level 2 (BSL-2) precautions for the production and use of NK cells based on NK/TIL cell expansion reagents. Below are some of the key precautions typically taken in BSL-2 laboratories:
- TRAINING: All laboratory staff must be trained in biosafety and understand the characteristics of the tumor cells being handled and the associated risks.
- Laboratory staff should wear appropriate personal protective equipment (PPE) such as lab coats, gloves, masks, goggles, or protective face shields.
- Biosafety Cabinets: Use biosafety cabinets for operations involving infectious materials to prevent the generation and spread of aerosols.
- Laboratory design: Laboratories should have clear entrances and exits, restricted access for unauthorized personnel, and hand-washing facilities.
- Waste Disposal: All infectious wastes should be placed in dedicated, biohazard-labeled containers that are sanitized and disposed of according to regulations.

- Disinfection of equipment and surfaces: Laboratory equipment and surfaces should be disinfected regularly to reduce the risk of cross-contamination.
- Sharps disposal: Use specialized sharps containers to collect needles, razor blades, and other sharp objects to avoid accidental injury.
- Operating Procedures: Follow strict operating procedures, such as avoiding contact with mouth suction when using pipettes, and avoiding eating, drinking, or using personal items in the work area.
- Emergency Plan: Develop and familiarize yourself with an emergency plan to respond to possible accidents, such as chemical spills, fires, or personal exposures.
- Health Surveillance: Laboratory staff should have regular health checks, especially for possible exposure to pathogens.
- Waste Decontamination: All potentially contaminated waste should be properly decontaminated before leaving the laboratory.
- Records and Reports: Maintain detailed laboratory records, including all experimental procedures and any accidents or exposure incidents.
- Safety Signs: Provide visible biohazard signs at laboratory entrances and exits to remind personnel of safety.

5. Steps to use the product

5.1 NK Cell Preparation Flowchart

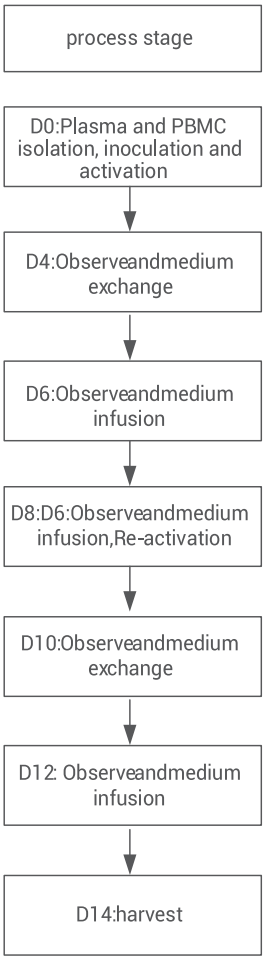

Figure 3. Flowchart of NK cell preparation

5.2 Procedure

5.2.1 D0 associated plasma collection, PBMC isolation and inoculation

5.2.1.1 Associated plasma collectiontion：Centrifuge fresh blood at 800g for 15min. Aspirate the upper layer of yellowish plasma into a 50mL centrifuge tube, inactivate in a water bath at 56 °C for 30min, and then centrifuge at 800g for 10min to remove the precipitate. Transfer the upper layer of plasma to a new 50mL centrifuge tube, add sodium heparin at the final concentration of 40IU/ml, mix well and store in a refrigerator at 4°C for use. The lower blood cell layer is used to extract peripheral blood mononuclear cells (PBMC).

5.2.1.2 Complete culture medium preparation：NK cell serum-free basal medium contains associated plasma (D0-D8:3%; D8-D10:1%; no addition after D10) and IL-2 at a final concentration of 400IU/mL.

5.2.1.3 Density gradient PBMC separation (Overlay Method)：Dilute the remaining lower blood cells with an equal volume of saline and mix well. Carefully and slowly pipet diluted blood on top of the Ficoll layer (diluted blood: Ficoll=2:1). Gently allow the Saline solution-diluted blood mixture to flow down the side of the bube and pool on top of the Ficoll layer surface with breaking the surface plane; slowly pipetting the blood mixture at a constant speed facilitates the layering in this step. Carefully cap the tubes.

5.2.1.4 Centrifuge at 400g for 30min at room temperature, speed up by 4, speed down by 3. Carefully aspirate the white membrane layer in the middle. Gently mix with saline. Centrifuge at 400g for 10min at room temperature. Discard the supernatant. Repeat the wash step once and discard the supernatant. Resuspend the cells with appropriate amount of complete culture medium and then take samples for counting.

5.2.1.5 Feeder cells thawing：thaw feeder cells in 37 °C water bath in 2 minutes. Transfer post-thaw feeder cells into the thawing solution (0.9% NaCl injection + 5% HSA), and centriffuge at 400g for 10min., Discard the supernatant and resuspend feeder cells with the appropriate amount of the complete culture medium. Centrifuge at 400g for 10min. Discard the supernatant and resuspend feeder cells with the appropriate amount of the complete culture medium. Take sample for cell counting.

5.2.1.6 Inoculation：According to the number of PBMC cells to be activated in culture and the ratio of feeder cells (recommended ratio of PBMC:feeder cells=1:2), take the corresponding volume of PBMC and feeder cells, and transfer into the appropriate culture vessel. Adjust the PBMC density at 2.5-5E5/ml by adding required volume of complete culture medium, and culture in a standard cell culture incubator at 37°C with 5% CO_2.

5.2.2 D4 Medium exchange

Observe cell morphology and status by microscopy and then conduct a two fold volume of medium exchange using the complete culture medium.

5.2.3 D6 Medium infusion

Observe cell morphology and status by microscopy. Conduct sampling and cell counting. Based on the cell counting result, readjust cell density to 3-5E5/mL using fresh complete culture medium.

5.2.4 D8 Re-activation and medium infusion

Observe cell morphology and status by microscopy. Conduct sampling and cell counting. Thaw a vial of cryopreserved feeder cells and follow 5.2.1.5, conduct feeder cell wash and counting for re-activation process. Based on the D8 cell counting result, calculate number of feeder cells to be used for re-activation (the ratio of NK:feeder cells = 1:1 is recommended). Transfer required number of feeder cells into the culture vessel and adjust the NK cell density to 2.5-5E5/mL using fresh complete culture medium.

5.2.5 D10 Medium exchange

Observe cell morphology and status by microscopy. Conduct sampling and cell counting. Based on the counting result, re-adjust the NK cell density to 5-8E5/mL using fresh complete culture medium.

5.2.6 D12 Medium infusion

Obsserve cell morphology and status by microscopy. Take sample for cell counting. Based on the counting result,re-adjust the NK cell density to 5-8E5/ml using fresh complete culture medium.

5.2.7 D14 Harvest

Observe cell morphology and status by microscopy., Take sample for counting. Based on the counting result, make decision on harvest or continuous culture. Harvest can be performed earlier or later based on the cell growth status and can be used directly or cryopreserved for future use..

Note: This NK cell culture protocol is for reference only. NK cell expansion culture status may vary due to individual differences in samples, culture mode adjustment and other factors. NK cell growth status can be adjusted appropriately through observation and analysis.

6. Trouble removal

Description of the problem	Possible causes	Solution
Is it normal to see significant clustering of NK cells in pre-culture? Is it necessary to intervene ?	The growth of cell clusters in the early stage indicates successful activation of NK cells, which is a normal phenomenon. Generally, around Day7, the clustered growth starts to ease and the cells start to grow in small clusters.	During the operation, please be careful not to blow the cells violently to avoid destroying the cell mass, so as not to affect the later expansion of NK cells.

7. Precautions for use

7.1 The use of sodium heparin anticoagulation tubes or sodium citrate blood collection bags is recommended for blood collection.

7.2 Associated plasma can affect the culture status of cells, and it is recommended that associated plasma may be reserved at about 30mL. Teh quality of the plasma may affect the expansion status of NK/TIL cells.

7.3 The reagent kit is limited for use in in vitro induction culture of NK/TIL cells. The components of different batches of the reagent kit cannot be used interchangeably.

7.4 If there is a crack in the outer tube of the reagent, stop using it and discard it properly.

7.5 The procedure should be carried out in a sterile environment, and it must be ensured that all reagent consumables used during the procedure that come into direct contact with the cytosol are strictly sterile.

7.6 This reagent must be used after opening, and must not be used after the freezing and thawing cycle.

7.7 Revive feeder cells properly follow the SOP provided. It is strictly prohibited to directly submerge the cryopreservation tubes containing the amplification reagents in the water bath during cell recovery, and it is recommended to ensure that at least the caps of the tubes are above the liquid level of the water bath.

7.8 Use of culture medium: warm basal medium naturally at room temperature and prepare the complete culture medium freshly each time before medium exchange/infusion.

7.9 Dispose spent medium and various bio-hazardous wastes generated during the experiment in accordance with the appropriate regulations and local regulatory requirements.

7.10 This NK/TIL Cell Expansion Reagent (Item No. HG-FEC001-RG) contains GFP labeling.

8. Related Products

Product name	Product No.
Blood/Tissue/Cell Genomic DNA Extraction Kit	HG-NA100
CAR/TCR Gene Copy Number Assay Kit (qPCR-Fluorescent Probe Method)	HG-CA001
RCL (VSVG) Gene Copy Number Assay Kit (qPCR-Fluorescent Probe Method)	HG-RC001
BaEV Gene Copy Number Assay Kit (qPCR-Fluorescent Probe Method)	HG-BA001
Mycoplasma DNA sample prep kit (magnetic bead method)	HG-CL200
Mycoplasma DNA Test Kit (qPCR-Fluorescent Probe Method)	HG-ZY002
Mycoplasma DNA Test Kit (qPCR-Fluorescent Probe Method)	HG-ZY001
CRS Cytokine ELISA Assay Kit	HG-HC001
HIV-1 p24 ELISA Assay Kit	HG-P001
Cellular Residual Human Interleukin 2 (IL-2) ELISA Assay Kit	HG-IL002
Cellular Residual Human Interleukin 7 (IL-7) ELISA Assay Kit	HG-IL007
Cellular Residual Human Interleukin 15 (IL-15) ELISA Assay Kit	HG-IL015
Cellular Residual Human Interleukin 21 (IL-21) ELISA Assay Kit	HG-IL021
Human Interferon gamma (IFN-γ) ELISA Assay Kit	HG-IF001
Viral E-hancer A	HG-PTD001-A
Viral E-hancer B	HG-PTD001-B
Viral E-hancer C（RUO）	HG-PTD001-C-R
Viral E-hancer C（GMP）	HG-PTD001-C-G
Viral E-hancer D	HG-PTD001-D
NK and TIL Cell Expansion Reagents（Feeder cell，GFP labeling）	HG-FEC001-RG
NK Cell Expansion Kit	HG-POC004
CD19 CAR-T Cell Preparation Kit	HG-POC001
Cell Killing Assay Kit (Suspending Target Cells)	HG-CKK001

9. Contact details

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