

Instructions for Use of NKG2D CAR-T Premade Lentivirus

Cat. No. HG-CT002

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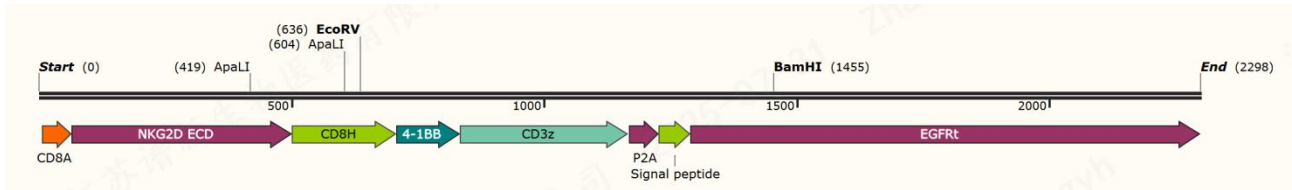
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Figure 1 (A) Schematic of the lenti-vector for anti-NKG2D CAR lentivirus production. The vector contains a kanamycin resistance gene. (B) Structural diagram of anti-NKG2D CAR components.

2. Applications

Viral Transduction Optimization Experiments.

Generation of NKG2D CAR-T Cells (For research use only, not for therapeutic purposes).

3. Product Specifications

Product Name	Catalog No.	Specification	Shelf Life	Storage
NKG2D CAR-T Premade Lentivirus	HG-CT002	0.5 mL/vial	24 months	-80°C

△CAUTION: Read instructions carefully and verify product information before starting experiments.

4. Storage

Lentivirus is shipped on dry ice. For long-term storage, maintain at -80° C. Avoid repeated freeze-thaw cycles, as titer may significantly decrease with each cycle.

△CAUTION: Avoid repeated freeze-thaw cycles for NKG2D CAR-T Premade lentivirus.

5. Biosafety

HIV-related genes (gag, pol, rev) are not expressed in transduced cells; they are expressed by packaging plasmids lacking the packaging signal. Despite low lentiviral replicative competence, handling requires a Biosafety Level 2 (BSL-2) facility. Wear lab coats, gloves, and eye/face protection. Avoid direct skin/eye contact. In case of inhalation, ingestion, or contact with skin, eyes, or clothing, handle immediately and wash thoroughly.

6. Required Reagents, Consumables, and Equipment

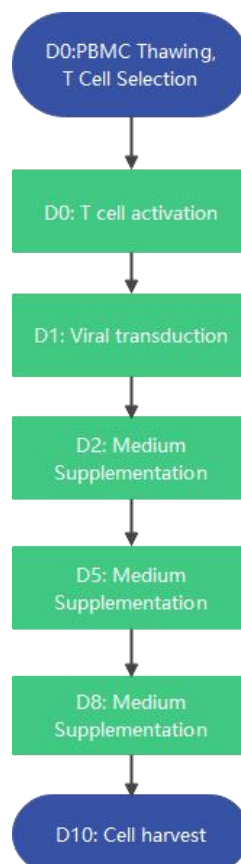
Prepare the following before experiments:

- ◆ Class II Biological Safety Cabinet

- ◆ CO₂ Incubator
- ◆ Pipettes (e.g., 1000 μL, 200 μL, 10 μL)
- ◆ Medical-grade -80° C Freezer
- ◆ Cell Counter
- ◆ Electric Thermostatic Water Bath
- ◆ Low-Adhesion Tips (1000 μL, 200 μL, 10 μL)
- ◆ PBMCs
- ◆ T Cell Medium
- ◆ T Cell Activation Reagents (Anti-human CD28 mAb, Anti-CD3 mAb)
- ◆ T Cell Transduction Enhancer
- ◆ CD3 Positive Selection Beads/Columns
- ◆ Human Interleukin-2 (IL-2)

7. Operating Procedures

7.1. Flowchart



7.2. Protocol

7.2.1. Complete T Cell Medium Preparation: Add 1 mL of 400,000 IU/mL IL-2 stock solution to 1 L T cell basal medium (final concentration: 400 IU/mL)

△CAUTION: Pre-warm complete medium to 37° C before each use to ensure optimal CAR-T expansion.

7.2.2. Day 0 (D0) Procedures:

PBMC Thawing: Thaw PBMCs in 37° C water bath. Wash with 5-10 volumes pre-warmed recovery medium. Centrifuge at 400g for 8 min. Discard supernatant. Resuspend pellet in pre-warmed complete medium and perform AO/PI counting.

T Cell Selection: Perform CD3 positive selection using magnetic beads on thawed PBMCs. Collect selected T cells.

T cell activation: Based on the counting results, take 4-5E7 T cells to be activated through a 5 mL pipette and transfer them to a 15 mL centrifuge tube. Cover the centrifuge tube with the lid and carefully transfer them to the corresponding adapter of the centrifuge. After balancing, centrifuge at room temperature for 400g, 10 minutes with an increase of 8 and a decrease of 8. After the centrifugation of T cells is completed, the clean supernatant is aspirated through a 5 mL pipette, and the complete culture medium is resuspended in a 10 mL pipette. The live cell density is adjusted to 1.5E6/mL, and the T cell solution is transferred to a T75 culture bottle. According to the volume of T cell activation, add the required amount of T cell activation reagent (recommended concentration for T cell activation reagent is 500 ng/mL). After mixing with a 10 mL pipette, cover the T75 culture bottle cap tightly and activate the culture in a 37 °C and 5% CO2 cell culture incubator for 24 ± 2 hours.

7.2.3. Day 1 (D1) Transduction:

Retrieve activated cells. Mix suspension in BSC. Transfer 150 μL to 1.5 mL tube for counting.

Remove the frozen lentivirus and Viral E-hancer B (T cell transduction enhancer B), and thaw them in a dark environment at 2-8 °C. After complete melting, wipe with a dust-free cloth and disinfect the surface before transferring into a biosafety cabinet. Open the packaging tube covers of lentivirus and Virus E-hancer B. According to the counting results, add the required amount of lentivirus through a 200 μ L pipette tip based on MOI (MOI recommended range is 0.5-2) and lentivirus titer (virus volume to be added μ L=total number of cells to be transduced MOI ÷ virus titer IU/mL 1000), and then add the required amount of Virus E-hancer B through a 1 mL pipette tip (add Virus E-hancer B according to the volume of transduction enhancer: cell suspension=1:100). After mixing with a 10 mL pipette, cover the T75 culture bottle cap tightly and let it stand in a 37 °C and 5% CO2 cell culture incubator for 24 ± 2 hours for transduction.

△CAUTION: During the process of cell blowing and mixing, the operation should be kept gentle to prevent excessive blowing force from causing a high proportion of cell death. The dosage mentioned in this instruction is recommended, and users can explore the dosage gradient according to their own process conditions. The virus transduction method is a suggested step, and users can choose according to their own process conditions.

7.2.4. Day 2 (D2) Medium Supplementation:

Add complete medium to adjust to 5×10⁵ viable cells/mL. Transfer to appropriate vessel (e.g., T175 flask if volume >50 mL). Culture at 37° C/5% CO₂ .

7.2.5. Day 5 (D5) Medium Supplementation:

Adjust to 5×10⁵ viable cells/mL with complete medium. Transfer to suitable vessel (e.g., cell culture bag). Culture.

7.2.6. Day 8 (D8) Medium Supplementation:

Adjust to 5×10^5 viable cells/mL. Transfer as needed. Culture.

7.2.7. Day 10 (D10) Harvest:

Observe cells, count, and harvest. Timing may be adjusted for immediate use or cryopreservation.

△CAUTION: This CAR-T protocol is a reference. Adjust based on cell growth observations due to sample variability or process modifications.

8. Troubleshooting

Problem	Possible source	Solution
Cell viability below 80%	The initial cell viability is poor, and the sorted T cells are in a poor state, resulting in lower viability in subsequent activation, transduction, and other processes	If the cell viability is below 70% before transduction, it is recommended to replace the cells and retest to avoid affecting subsequent test performance and results.
	During the process of virus transduction, the need to penetrate the cell membrane and enter the nucleus can cause certain damage to the cells, which is a normal phenomenon	Continuing to observe the culture, in general, the cell viability will decrease to varying degrees on the second day after virus transduction, with viability generally ranging from 60% to 85%. Starting from the third day, the cells will gradually recover and viability will begin to increase.
	The toxicity of the virus itself	Due to factors such as virus packaging preparation process and sequence design, the virus may be highly toxic and cause significant cell damage. Therefore, optimizing the sequence or virus preparation process can be considered.
	The cell culture system needs to be optimized	Due to the insufficient support provided by the culture system for cell recovery and proliferation, it is necessary to optimize and explore the culture system.

9. Buyer Notice

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Attachment 1: Related Products (For more products, please consult Hillgene)

<https://www.hillgene.com>)

Product Name	Catalog No.
Blood/Tissue/Cell Genomic DNA Extraction Kit	HG-NA100
CAR/TCR Gene Copy Number Detection Kit (Multiplex qPCR)	HG-CA001
RCL (VSVG) Gene Copy Number Detection Kit (qPCR)	HG-RC001
Mycoplasma DNA Sample Preprocessing Kit (Magnetic Bead Method)	HG-CL200
Mycoplasma DNA Detection Kit (qPCR)	HG-ZY002
Viral E-hancer B	HG-PTD001-B
CD-19 CAR-T Premade Lentivirus	HG-CT1901
CD-19 CAR-NK Premade Lentivirus	HG-CN1901
NKG2D CAR-T Premade Lentivirus	HG-CT002
IL15-CD19 Premade Lentivirus	HG-CN02-IL15
IL15 Premade Lentivirus	HG-CN1501
HIV-1 p24 ELISA Detection Kit	HG-P001
Human IFN- γ ELISA Detection Kit	HG-IF001
Cell Cytotoxicity Assay Kit (Suspended Target Cells)	HG-CKK001

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