Hillgene

让细胞药物谱写生命新篇章

CELL THERAPY INNOVATION INSPIRED

Ver202507

Instructions for Use of IL15 Premade Lentivirus

Cat. No. HG-CN1501

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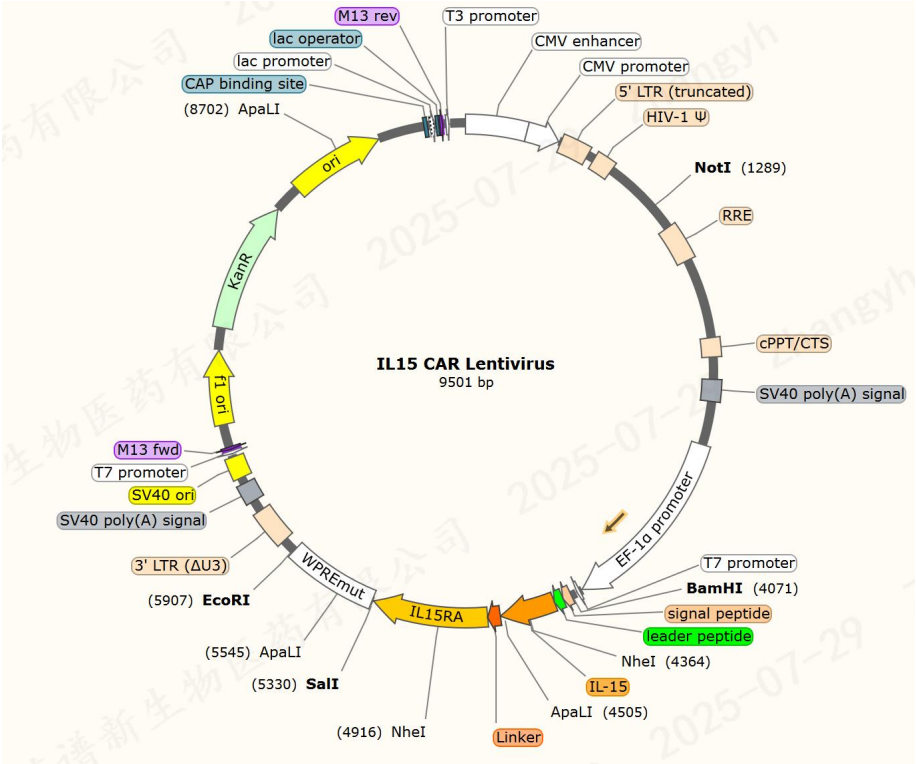
1. Product Introduction

As a critical cytokine for enhancing NK cell persistence, wild-type IL-15 is a short half-life secretory protein. Our NK product engineers IL-15 into a membrane-bound protein, effectively extending its longevity while restrictively targeting its effect to the therapeutic NK cells carrying it. Furthermore, utilizing IL-15 receptor alpha (IL-15Rα) for membrane tethering not only prolongs IL-15's half-life and restricts its function protectively but also overcomes the limitations of IL-15 signal trans-presentation.

Hillgene has developed an IL-15 CAR-NK lentivirus with specific functionality and convenient transduction efficiency identification based on second-generation CAR-NK technology. This virus utilizes the BaEV envelope protein, enabling transduction of most mammalian cells, including primary cells and non-dividing cells.

This virus is produced using the Hillgene platform with serum-free medium and suspension culture technology and **293T cell line** for upstream packaging system and downstream lentiviral purification system, resulting in a high-quality, Premade IL-15 CAR-NK lentivirus product with high viral titer and low impurity content.

A



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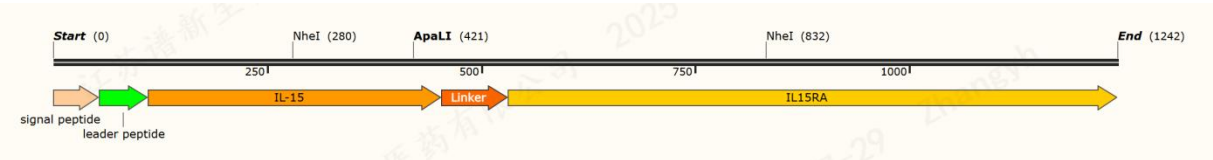


Figure 1 (A) Schematic diagram of the lenti-vector used to produce the IL-15 CAR-NK lentivirus. The vector contains a kanamycin resistance gene. (B) Structural diagram showing the IL-15 CAR components.

2. Applications

Viral Transduction Optimization Experiments.

Production of Engineered Cytokine-Expressing NK Cells (For research use only, not for therapeutic purposes).

3. Product Specifications

Product Name	Catalog No.	Specification	Shelf Life	Storage
IL15 Premade Lentivirus	HG-CN1501	0.5 mL/vial	24 months	-80℃

△**CAUTION: Please read the instructions carefully and verify product information before starting experiments.**

4. Storage

Lentivirus is shipped on dry ice. For long-term storage, it is recommended to store the virus at -80° C. Avoid repeated freeze-thaw cycles. Titer may decrease significantly with each freeze-thaw cycle.

△**CAUTION: Avoid repeated freeze-thaw cycles when using the IL-15 CAR-NK 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. Although lentiviruses have low replication competence, they require handling in a **Biosafety Level 2 (BSL-2) facility**. For your safety and health, wear protective garments, gloves, and eye covering and face masks during operation, and avoid direct contact of the lentiviral product with skin and eyes. In case of inhalation, ingestion, skin or eye contact, or contact with clothing, handle immediately and wash thoroughly.

6. Required Reagents, Consumables, and Equipment

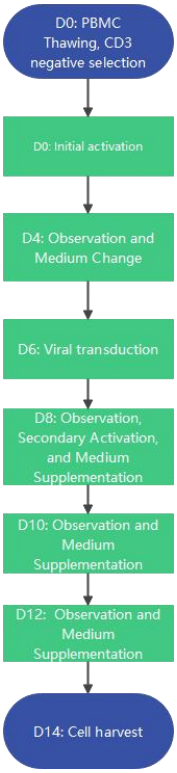
Please prepare the following reagents, consumables, and equipment before starting the experiment:

- Class II Biological Safety Cabinet
- CO_2 Incubator
- Pipettes of various sizes (e.g., 1000 µL, 200 µL, 10 µL)
- Medical-grade Low-temperature Freezer
- Cell Counter
- Electric Thermostatic Water Bath
- 1000 µL, 200 µL, 10 µL Low-Adhesion Pipette Tips

- PBMC (Peripheral Blood Mononuclear Cells)
- NK Cell Culture Medium
- CD3 Negative Selection Magnetic Beads/Columns
- K562 Feeder Cells
- Human Interleukin-2 (IL-2)

7. Operating Procedures

7.1. Flowchart



7.2. Operating Steps (Feeder-Based Process)

7.2.1. Preparation of Complete NK Cell Culture Medium: NK Cell Medium + Serum Replacement SR (1%–10%) + IL-2 (200–600 IU/mL).

7.2.2. Day 0 (D0) Procedures:

PBMC Thawing: Thaw PBMCs in a 37° C water bath. Add 5–10 volumes of pre-warmed recovery medium to wash the PBMCs. Centrifuge at 400g for 8 minutes, discard supernatant. Resuspend the pellet in pre-warmed complete medium, mix well, and take a sample for AO/PI counting.

CD3 Negative Selection: Perform CD3 negative selection using magnetic beads on the thawed PBMCs. Collect the negatively selected cells.

K562 Feeder Cell Thawing: Thaw K562 feeder cells in a 37° C water bath. Place thawed cells in an appropriate volume of medium. Centrifuge at 400g for 10 minutes to wash, discard supernatant. Resuspend in an appropriate volume of complete NK cell medium, mix well, centrifuge again at 400g for 10 minutes, discard supernatant. Add an appropriate volume of complete NK cell medium, resuspend well, and take a sample for counting.

Initial Activation: Based on the counts of CD3-negative selected cells and K562 feeder cells, mix them at a specific ratio (CD3- cell count: K562 feeder cell count = 1:1, 1:2, or 1:4; adjust based on customer's process). Transfer the mixture to a suitable culture vessel. Add complete medium to adjust the cell culture density to 1-5 × 10^5 cells/mL. Culture at 37° C, 5% CO_2.

7.2.3. Day 4 (D4) Observation and Medium Change:

Observe cells and perform a medium change by adding twice the volume of fresh complete NK cell medium.

7.2.4. Day 6 (D6) Viral Transduction

Take a sample for AO/PI counting. Centrifuge the remaining cells at 400g for 8 min, discard supernatant (medium change), and proceed to transduction. Transduction MOI = 2-10, transduction density = 5×10^5 - 2×10^6 cells/mL.

Based on the total volume of the cell suspension after adding the viral vector, add Viral E-hance C at a volume ratio of 1:100 (Transduction Enhancer: Cell Suspension). Mix gently by pipetting slowly. Place cells in a 37° C, 5% CO_2 incubator and continue culturing for 24-48 hours.

Remove the transduced cell suspension. Centrifuge at 400g for 8 min at room temperature. Discard supernatant. Resuspend the cell pellet in complete medium. Adjust the cell density to 2-5 × 10^5 cells/mL. Place in a 37° C, 5% CO_2 incubator. Supplement medium every 2-3 days, adjusting the cell density to 2-5 × 10^5 cells/mL.

7.2.5. Day 8 (D8) Observation, Secondary Activation, and Medium Top-up:

Observe cells, take a sample for counting. Add the corresponding volume of K562 feeder cells at a specific ratio (recommended NK cell count: K562 feeder cell count = 1:1, 1:2, or 1:4; adjust based on customer's process). Add complete NK cell medium to adjust the cell density to 2-5 × 10^5 cells/mL.

7.2.6. Day 10 (D10) Observation and Medium Top-up:

Observe cells, take a sample for counting. Add complete NK cell medium based on cell concentration to adjust the density to 2-5 × 10^5 cells/mL.

7.2.7. Day 12 (D12) Observation and Medium Top-up:

Observe cells, take a sample for counting. Add complete NK cell medium based on cell concentration to adjust the density to 2-5 × 10^5 cells/mL.

7.2.8. Day 14 (D14) Cell Harvest:

Observe cells, take a sample for counting, and harvest the cells. Harvest timing can be adjusted earlier or later based on experimental needs for immediate use or cryopreservation.

CAUTION: This NK cell culture procedure is for reference only. Due to individual sample differences, adjustments in culture methods, and other factors, the expansion status of NK cells may vary. Observe and analyze the growth status of the NK cells and make appropriate adjustments if necessary.

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 contact Hillgene at 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
BaEV Gene Copy Number Detection Kit (qPCR)	HG-BA001
Mycoplasma DNA Sample Preprocessing Kit (Magnetic Bead Method)	HG-CL200
Mycoplasma DNA Detection Kit (qPCR)	HG-ZY002
Viral E-hancer C	HG-PTD001-C-R
CD-19 CAR-T Of-The-Shelf Lentivirus	HG-CT1901
CD-19 CAR-NK Of-The-Shelf Lentivirus	HG-CN1901
NKG2D CAR-T Of-The-Shelf Lentivirus	HG-CT002
IL15-CD19 Of-The-Shelf Lentivirus	HG-CN02-IL15
IL15 Of-The-Shelf Lentivirus	HG-CN1501
Genetically Modified K562 Feeder cell	HG-FEC002-RU-1
	HG-FEC002-RU-4
HIV-1 p24 ELISA Detection Kit	HG-P001
Residual K562 feeder cell Detection Kit	HG-KF001
Cell Cytotoxicity Assay Kit (Suspended Target Cells)	HG-CKK001

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