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Instructions for Use of

CD19 CAR-NK Premade Lentivirus

Cat.No. HG-CN1901

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1. Product Introduction

CD19 (also known as Cluster of Differentiation 19, B-lymphocyte surface antigen B4, or CVID3) is a glycoprotein expressed on the surface of B lymphocytes during most stages of B-cell maturation. It is strictly required for the terminal differentiation of B cells. Mutations in the CD19 gene lead to severe immunodeficiency syndromes with impaired antibody production, such as CVID3 (Common Variable Immunodeficiency 3). Most B-cell malignancies express normal to high levels of CD19, making it a nearly ideal target for cancer immunotherapy.

Hillgene has developed a CD19 CAR-NK lentivirus with specific killing 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. The virus employs a second-generation CAR structure design, incorporating an anti-CD19 (clone FMC63) ScFv portion, CD8 hinge, 4-1BB costimulatory domain, and CD3Z signaling domain. This design allows CAR-NK cells to survive longer and promotes cell proliferation. Additionally, the virus has been modified for KanR (kanamycin resistance) to meet domestic and international regulatory requirements.

This virus is produced using the Puxin platform's serum-free suspension packaging system and downstream lentiviral purification system (293T cell line), resulting in a high-quality, off-the-shelf CD19 CAR-NK lentivirus product with high viral titer and low impurity content. With this product, customers can bypass the complex and tedious process of lentiviral vector preparation for **cell process optimization and validation**, and can also use this product as a **positive control to guide cell process development**, accelerating the development workflow.

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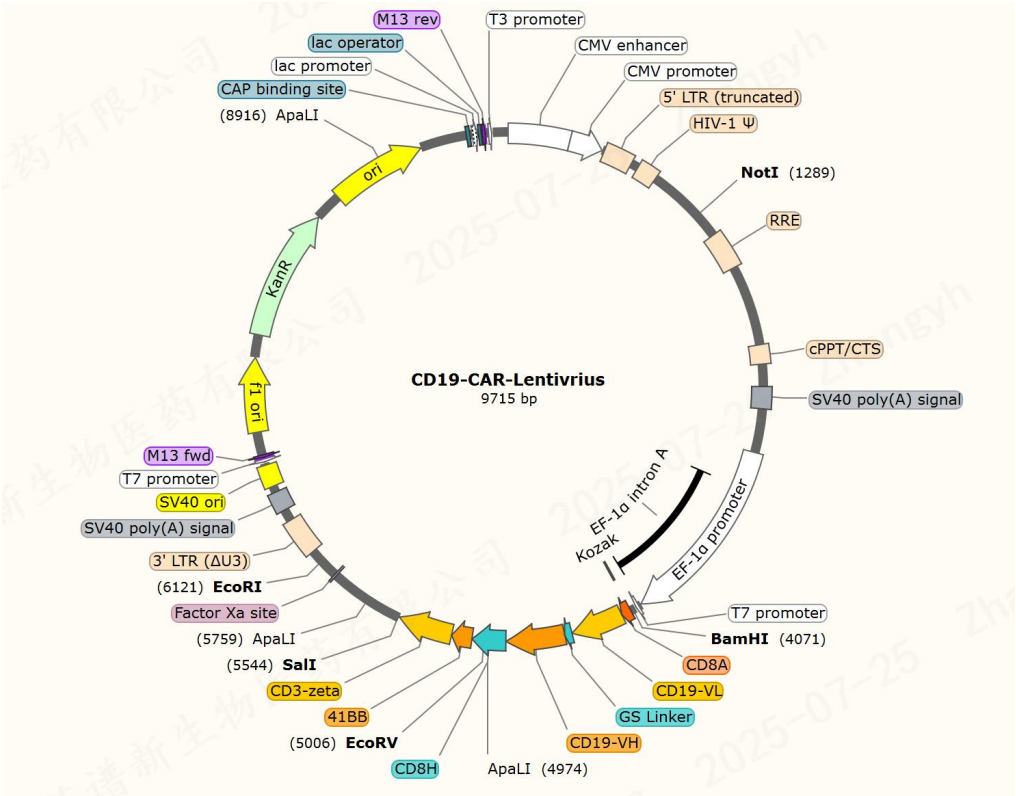


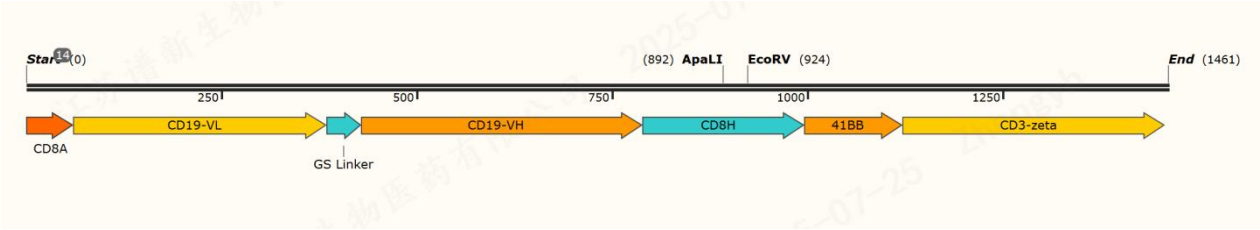


Figure 1 (A) Schematic diagram of the lenti-vector used to generate the anti-CD19 CAR lentivirus. The vector contains a kanamycin resistance gene. (B) Structural diagram showing the anti-CD19 CAR components.

2. Applications

Positive control for evaluating anti-CD19 CAR in NK cells.

Viral Transduction Optimization Experiments.

Generation of Anti-CD19 CAR-NK Cells(For research use only, not for therapeutic purposes).

3. Product Specifications

Product Name	Product Catalog	Specification	Shelf Life	Storage
CD19 CAR-NK Premade Lentivirus	HG-CN1901	0.5mL/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 CD19 CAR-NK Off-the-Shelf 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 a lab coat, gloves, and eye/face protection 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

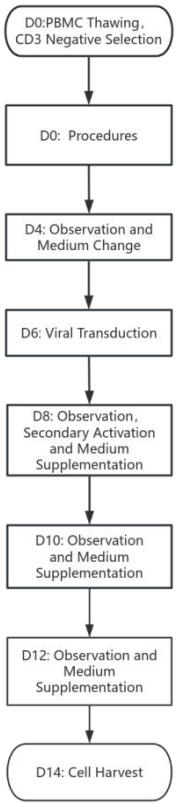
Please prepare the following reagents, consumables, and equipment before starting the experiment:

- 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, 10μLLow-Adhesion Pipette Tips
- PBMC
- NK Cell Culture Medium
- CD3 Negative Selection Magnetic Beads/Columns
- K562 feeder cell
- 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 mixed CD3-negative selected cells and K562 feeder cells to a suitable culture vessel. Add complete medium to adjust the cell culture density to 1-5 x 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 = $5x10^5$ - $2x10^6$ 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 x 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 x 10^5 cells/mL.

7.2.5.Day 8 (D8) Observation, Secondary Activation, and Medium Supplementation:

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 x 10^5 cells/mL.

7.2.6.Day 10 (D10) Observation and Medium Supplementation:

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

7.2.7. Day 12 (D12) Observation and Medium Supplementation:

Observe cells, take a sample for counting. Add complete NK cell medium based on **medium color change or** cell concentration to adjust the density to 2-5 x 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 CAR-NK cell culture procedure is for reference only. Due to individual sample differences, adjustments in culture methods, and other factors, the expansion status of CAR-NK cells may vary. Observe and analyze the growth status of the CAR-NK cells and make appropriate adjustments if necessary.

8. Troubleshooting

Problem	Possible Cause	Solution
Cell viability <80%	Poor initial cell viability and suboptimal condition of the sorted NK cells lead to low viability during subsequent activation and transduction steps.	If the cell viability is below 70 % prior to transduction, replace the cells and repeat the test to avoid compromising downstream performance and results.
	During viral transduction, the virus must cross the plasma membrane and enter the nucleus, inevitably causing some cellular damage; this is considered normal.	Continue monitoring the culture. Typically, cell viability declines to 60 – 85 % on the second day after viral transduction, then gradually recovers and begins to rise from the third day onward.
	Intrinsic viral cytotoxicity.	Viral packaging methods or sequence design may confer high toxicity and strong cellular damage; optimize the sequence or the viral production process.
	The CAR-NK cell culture system needs optimization.	The current culture system may not supply adequate nutrients for CAR-NK cell recovery and expansion; optimize and empirically refine the culture conditions.

9. Buyer Notice

Our products are for research use only. They must not be used for any other purposes, including, but not limited to, use in humans, therapeutic or diagnostic applications, or any commercial use. Without our consent, our products may not be transferred to third parties, resold, modified for resale, used to manufacture commercial products, or used to provide services to third parties.

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For additional information on products, intellectual property, and restricted use, please visit our website. https://www.hillgene.com。

Attachment 1: Related products

(For additional products, please contact Hillgene at https://www.hillgene.com)

Product Name	Product Catalog
Genomic DNA Extraction Kit for Blood Tissue Cells	HG-NA100
CAR/TCR Gene Copy Number Detection Kit (Multiplex qPCR)	HG-CA001
BaEV Gene Copy Number Detection Kit(qPCR)	HG-BA001
Mycoplasma Residual DNA Sample Pretreatment Kit (Magnetic Bead Method)	HG-CL200
Mycoplasma DNA Detection Kit (qPCR)	HG-ZY002
Viral E-hancer C	HG-PTD001-C-R
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
Cytotoxicity Assay Kit (Suspension Target Cells)	HG-CKK001

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