Ver202507

Instructions for Use of

CD19 CAR-T Premade Lentivirus

Cat.No. HG-CT1901

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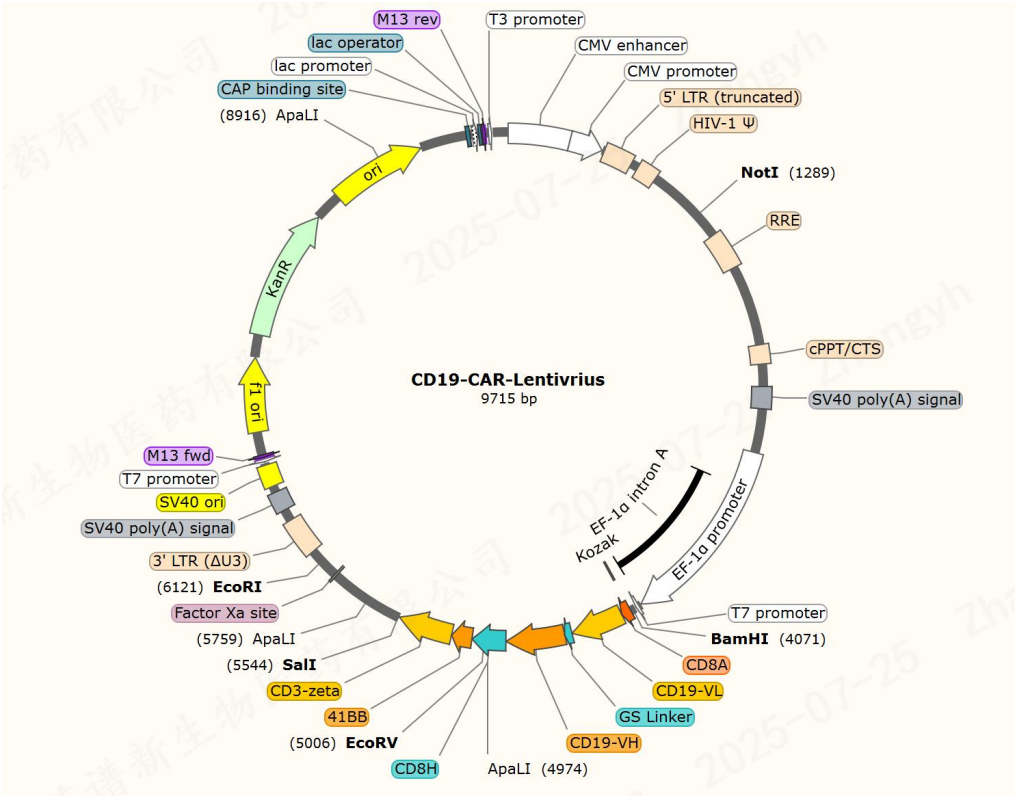
1. Product Introduction

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.

Puxin Biotech has developed a CD19 CAR-T lentivirus with specific killing functionality and convenient transduction efficiency identification based on second-generation CAR-T technology. This virus utilizes the VSV-G 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-T 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 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.

A



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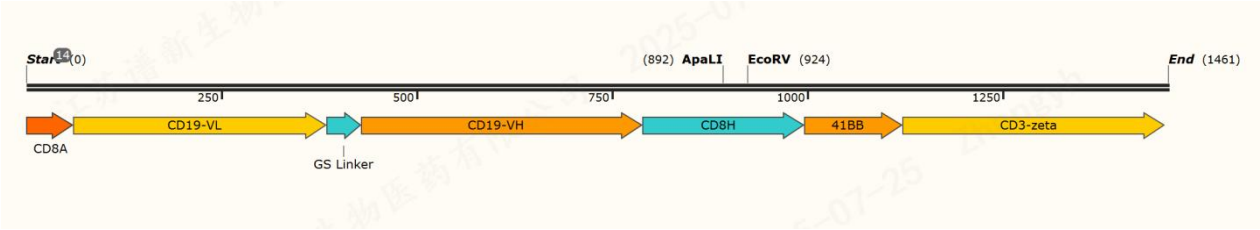


Figure 1 (A) Schematic diagram of the lenti-vector used to generate the anti-CD19 CAR lentivirus. The vector contains a kanamycin resistance gene. (B) Structural diagram showing the anti-CD19 CAR components.

2. Applications

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

Viral Transduction Optimization Experiments.

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

3. Product Specifications

Product Name	Product Catalog	Specification	Shelf Life	Storage
CD19 CAR-T Premade Lentivirus	HG-CT1901	0.5mL/vial	24months	-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-T 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

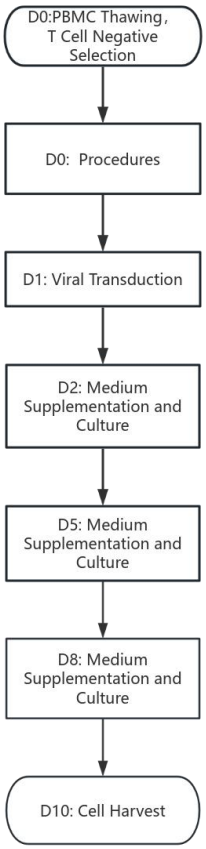
Please prepare the following reagents, consumables, and equipment before starting the experiment:

- 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, 10μL Low-Adhesion Pipette Tips
- PBMC
- T Cell Culture Medium
- T Cell Activation Reagents (Anti-human CD28 mAb、Anti CD3 mAb)
- T Cell Transduction Enhancer B
- CD3 Positive Selection Magnetic Beads/Columns
- Human Interleukin-2 (IL-2)

7. Operating Procedures

7.1. Flowchart



7.2. Operating Steps

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

△CAUTION: To ensure optimal CAR-T cell expansion, pre-warm the prepared complete medium to 37° C before each use.

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.

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

T Cell Activation: Based on cell count, transfer 4-5 × 10^7 T cells to a 15 mL centrifuge tube using a 5 mL pipette.

Cap the tube, carefully transfer to a centrifuge adapter, balance, and centrifuge at 400g for 10 min at room temperature (acceleration setting 8, deceleration setting 8). After centrifugation, carefully aspirate the supernatant using a 5 mL pipette.Resuspend cells in complete medium using a 10 mL pipette and **adjust viable cell density to 1.5 × 10^6 cells/mL.Transfer the T cell suspension to a T75 flask. Add the required volume of T Cell Activation Reagents (**recommended concentration: 500 ng/mL**).Mix gently by pipetting with a 10 mL pipette. Cap the flask tightly and culture at 37° C, 5% CO_2 for 24 ± 2 hours.

7.2.3.Day 1 (D1) Viral Transduction Procedures:

Remove activated cells (24 ± 2h) from the incubator.In the biosafety cabinet, open the T75 flask and mix gently by pipetting with a 10 mL pipette.Transfer 150 µL of cell suspension to a 1.5 mL tube using a 200 µL pipette tip for cell counting.

Thaw the frozen lentivirus and **Viral E-hancer B (T Cell Transduction Enhancer B)** protected from light at 2-8° C.After complete thawing, wipe with lint-free cloth, surface disinfect, and transfer into the biosafety cabinet.Open the caps of the lentivirus and Viral E-hancer B vials.Based on cell count, add the required volume of lentivirus using a 200µL pipette tip (**recommended MOI range: 0.5-2**; volume in µL = total cells × MOI ÷ viral titer IU/mL × 1000).Add the required volume of **Viral E-hancer B** using a 1 mL pipette tip (add at a volume ratio of 1:100 [Transduction Enhancer : Cell Suspension]).Mix gently by pipetting with a 10 mL pipette. Cap the flask tightly and incubate statically at 37° C, 5% CO_2 for 24 ± 2 hours.

△CAUTION:Handle cells gently during pipetting to prevent excessive cell death due to forceful pipetting.The amounts stated in this protocol are recommendations. Users may optimize gradients based on their specific processes.The viral transduction method is a suggested step. Users may choose alternatives based on their specific processes.

7.2.4.Day 2 (D2) Medium Supplementation and Culture:

Based on cell count, add complete medium using a 10 mL pipette to adjust viable cell density to 5 × 10^5 cells/mLTransfer cells to an appropriate culture vessel based on volume (e.g., T175 flask if volume >50 mL). Cap tightly and culture at 37° C, 5% CO_2

7.2.5.Day 5 (D5) Medium Supplementation and Culture:

Based on cell count, add complete medium to **adjust viable cell density to 5 × 10^5 cells/mL.Transfer cells to an appropriate culture vessel based on volume (e.g., cell culture bag). Culture at 37° C, 5% CO_2 .

7.2.6.Day 8 (D8) Medium Supplementation and Culture:

Based on cell count, add complete medium to **adjust viable cell density to 5 × 10^5 cells/mL. Transfer cells to an appropriate culture vessel based on volume (e.g., cell culture bag). Culture at 37° C, 5% CO_2 .

7.2.7. Day 10 (D10) 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-T cell culture procedure is for reference only. Due to individual sample differences, adjustments in culture methods, and other factors, the expansion status of CAR-T cells may vary. Observe and analyze the growth status of the CAR-T 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 T 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 cell culture system needs optimization.	The current culture system may not supply adequate nutrients for cell recovery and expansion; optimize and empirically refine the culture conditions.

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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
RCL (VSVG) Gene Copy Number Detection Kit (qPCR)	HG-RC001
Mycoplasma Residual DNA Sample Pretreatment Kit (Magnetic Bead Method)	HG-CL200
Mycoplasma DNA Detection Kit (qPCR)	HG-ZY002
Viral E-hancer B	HG-PTD001-B
CD19 CAR-T Off-the-Shelf Lentivirus	HG-CT1901
CD19 CAR-NK Off-the-Shelf Lentivirus	HG-CN1901
NKG2D CAR-T Off-the-Shelf Lentivirus	HG-CT002
IL-15 Off-the-Shelf Lentivirus	HG-CN02-IL15
IL-15 Off-the-Shelf Lentivirus	HG-CN1501
HIV-1 p24 ELISA Detection Kit	HG-P001
Human IFN-γ ELISA Detection Kit	HG-IF001
Cytotoxicity Assay Kit (Suspension Target Cells)	HG-CKK001

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邮　箱：info@hillgene.com
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