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Instructions for Use of CD19 CAR-T Cell Preparation Kit

Cat.No. HG-POC001

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

CONTENTS

1. Product Description

CD19 CAR-T cell preparation kit is composed of three parts: T cell activation and expansion unit, T cell purification unit and CD19 CAR gene transduction unit. The T cell activation and expansion unit consists of T cell basal medium and T cell activation reagent, which provides nutrients for T cell culture, and the T cell activation reagent provides T cell activation signals for T cell activation; the T cell purification unit consists of T cell sorting beads and columns, which is capable of obtaining high purity T cells from single blood collection or PBMC; The CD19 CAR gene transduction unit contains CD19 CAR-T spot lentivirus and T cell transduction enhancer (Viral E-hancer B), which enables efficient integration of CD19 CAR sequence. This kit is easy to operate, and prepares CAR-T cells with high expansion multiplicity and cell viability, CAR gene transduction efficiency and T cell purity. This product is for in vitro research use only.

2. Production Composition

Product name	Cat.No	Composition	Cat.No	Specification	Amount	preservation condition
CD19 CAR-T Cell Preparation Kit	HG-POC001	T cell basal medium	HG-POC001-04	1000 mL/bottle	two bottles	2~8°C
		T cell activation reagent	HG-POC001-03	20 μL/vial	one stick	2~8°C
		CD19 CAR-T spot lentivirus	HG-CT1901	0.5 mL/vial	one stick	-80°C
		T-cell transduction enhancer（Viral E-hancer B）	HG-PTD001-B	0.2 mL/vial	Two sticks	
		CD3 sorting beads	HG-POC001-02	0.1 mL/vial	one stick	2~8°C
		Cell sorting column	HG-POC001-05	stick	one stick	

Individual components of the CD19 CAR-T Cell Preparation Kit should be stored according to the preservation conditions in the table above, and their products are valid for 36 months.
Starting cell is about 4-5E7, 100-fold expansion, harvested cell is 4-5E9.

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

Serial number	Name of reagent and consumables	Dosage
1	T cell basal 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	4 stick
8	T75 flask	1~2 stick
9	T175 flask	1~2 stick
10	825 cm^2 cell culture bag	1 stick
11	15 mL centrifuge tube	a certain number or amount
12	1.5 mL centrifuge tube	a certain number or amount
13	10 mL、25 mL pipette	a certain number or amount
14	1mL 和 200 μL pipette tips	a certain number or amount

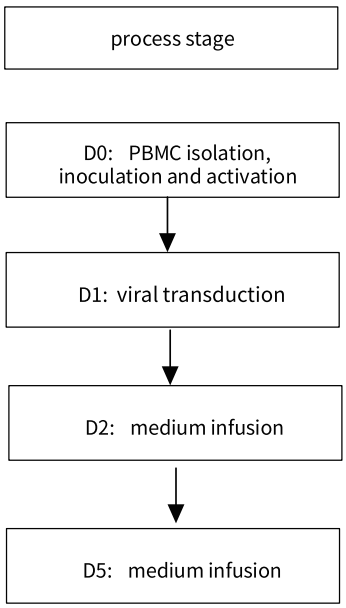
4. General considerations and biosafety guidelines

Please follow Biosafety Level 2 (BSL-2) precautions for the production and use of CD19 CAR-T Cell Preparation Kit 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. Usage and operation steps

5.1. CD19-CAR-T Cell Preparation Flowchart



5.2. T-cell complete medium preparation: T-cell basal medium + IL-2 (400IU/mL).

5.3. D0 plasma, PBMC isolation and inoculation

5.3.1. Separate PBMC: mix equal volume of saline and peripheral blood, slowly add it to the Ficoll layer (diluted peripheral blood: Ficoll=2:1) to keep the stratification clear, centrifuge at 400g for 30 minutes at room temperature, increasing speed 4, decreasing speed 3, then carefully aspirate the white membrane layer in the middle, add saline to mix well, centrifuge at 400g for 10 minutes at room temperature, discard the supernatant, and repeat washing once again for PBMC, discard the supernatant, resuspend the cells with appropriate T-cell complete medium and take samples for counting. The supernatant was discarded, and the washing of PBMC was repeated once again, the supernatant was discarded, and the cells were resuspended with appropriate amount of T-cell complete medium and then sampled and counted.

5.3.2. T cell inoculation and activation: According to the cell counting results, the 4E7 sorted T cells were used for inoculation, the T cell complete medium was replenished to 40mL, and 20μL of T cell activation reagent was added and mixed well, then inoculated in T75 culture flasks, and activation culture was carried out at 37°C with 5% CO_2.

5.4. D1 viral transduction

Cells were observed, mixed and sampled for viable cell counting, centrifuged at 400g for 10 minutes after counting, cell precipitates were resuspended with appropriate amount of T-cell complete medium, CD19 CAR-T spot lentivirus was added according to the cell number according to the MOI=2, and the T-cell complete medium was replenished to 2E6 cells/mL, inoculated in six-well plates, centrifuged at 1000g for 30 minutes, and activated in culture at 37°C with 5% CO_2. Activation culture was performed at 37°C , 5% CO_2.

5.5. D2 medium infusion

Cells were observed, mix well and take samples for viable cell counting, according to the counting result resuspend with appropriate amount of T cell complete medium and adjust the cell density to 5E5/mL, according to the cell resuspension volume inoculate in T75/T175 culture flasks, and incubate at 37°C , 5% CO_2.

5.6 D5 medium infusion

Cells were observed, mixed and sampled for viable cell counting, and fresh T cell complete medium was

replenished according to the viable cell count to viable cell density to 5E5/mL, inoculated into T175 culture flasks according to the cell volume, and cultured at 37°C with 5% CO_2.

5.7 D8 medium infusion

Cells were observed, and samples were taken for viable cell counting after mixing. Fresh T cell complete medium was supplemented according to the number of viable cells to the density of viable cells to 5E5/mL, inoculated in 825 cm^2 cell culture bags, and cultured at 37°C with 5% CO_2.

5.8 D10 Harvesting and Cryopreservation

The cells were observed, mixed and sampled for viable cell counting, and according to the counting results, samples were sent for CD3 positivity and CD19 CAR positivity. The remaining cells were centrifuged at 400g for 10 minutes, the cell precipitate was resuspended with appropriate amount of wash Buffer and then centrifuged at 400g for another 10 minutes, the cell precipitate was resuspended with cryopreservation solution and then dispensed into cryopreservation tubes.

6. Trouble removal

Description of the problem	Possible causes	Solution

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. This reagent is intended for use in obtaining CD19 CAR-T cells only, and reagent components from different batches cannot be used interchangeably.

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

7.4. 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.

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

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

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

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

9. Contact details

Address: Building 4, Yue Wang Park, No. 1463, Wuzhong Avenue, Wuzhong District, Suzhou , Jiangsu, China.
Postal code：215104
Tel：400-900-1882
E-Mail：info@hillgene.com
Website：https://www.hillgene.com

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Jiangsu Hillgene Biopharma Co.,Ltd

A : Building 4, Yue Wang Park, No. 1463, Wuzhong Avenue, Wuzhong District, Suzhou , Jiangsu, China.

T : 400-900-1882

E : info@hillgene.com

W : www.hillgene.com/www.bluekitbio.com

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