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Instructions for Use of Viral E-hancer A &Viral E-hancer B

(T Cell Specific)

CONTENTS

1. Product description

Viral E-han cer is a series of efficient, non-cytotoxic viral transduction enhancers, and Viral E-han cer is suitable for viral transduction of T cells, NK cells as well as HSC cells, depending on different mechanisms of action. Viral E-hancer improves the efficiency of virus transduction by reducing the cell membrane viscosity, improving lipid exchange and transmembrane transport, and temporarily regulating the expression of some protein on the cell surface. Among these, the combination of Viral E-hancer A and Viral E-hancer B provided a significant boost to virus-transduced T cells.

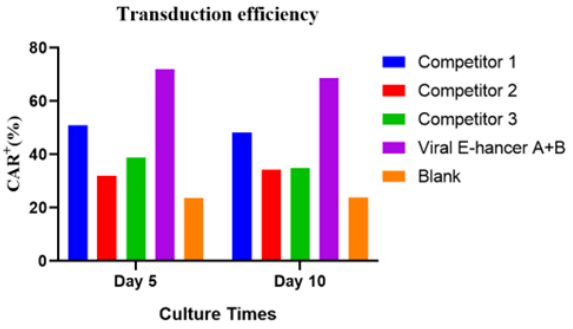

Figure 1 Comparison of transduction efficiency of Viral E-hancer A & Viral E-hancer B at T cell level (MOI = 0.5)

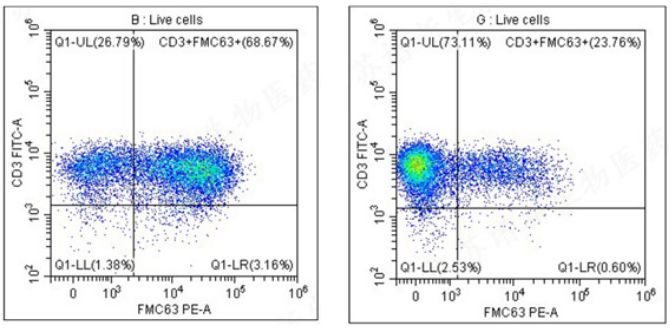

Figure 2 Left : CAR T cell flow with Viral E-hancer A & Viral E- hancer B combination ; Right : CAR T cell flow without transduction enhancer

2. Product components

Product name	Art.No	Specifications	Storage
Viral E -hancer A	HG-PTD001-A	283.7μ M : 0.2 mL	-20°C Fridge
Viral E -hancer B	HG-PTD001-B	10mg /ml : 0.2 mL	-20°C Fridge

2.1. If there are cracks in the reagent packaging tube, it should be stopped immediately.

2.2. The operation process shall be carried out in a sterile environment, and all direct contact test agent consumables used during the operation must be strictly sterile.

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

Number	Name of the reagent consumables	Amount (eg. six-well plate)
1	Virus	As needed
2	Culture medium	As needed
3	Six well plate	1
4	T25 flask	1
5	T75 flask	2
6	T175flask	2
7	15mL centrifuge tube	Several
8	50mL centrifuge tube	Several
9	10mL pipette	Several
10	25mL pipette	Several
11	1mL pipette tips	Several
12	200μL pipette tips	Several

4. General considerations and biosafety guidelines

- Please use the Virus E-hancer series products in accordance with Biosafety Level 2 (BSL-2) protection measures. Here are some of the key precautions commonly taken in BSL- 2 laboratories :
- Training : All laboratory staff must be trained on biosafety knowledge about the characteristics and relevance of the tumor cells treated The risk of.
- Laboratory staff should wear appropriate personal protective equipment such as laboratory clothing, gloves, masks, goggles or protective masks.
- Biosafety cabinet : Use the operation of infectious materials to prevent the generation and spread of aerosols.
- Laboratory design : The laboratory shall have clear entrance and exit, restricted access to unauthorized personnel, and be equipped with handwashing facilities.
- Waste disposal: All infectious waste should be placed in dedicated containers with biological hazard labels and disinfected and treated according to regulations..
- Equipment and surface disinfection : Regularly disinfect laboratory equipment and surfaces to reduce the risk of cross-contamination.
- Sharp processing : use a special sharp box to collect needles, blades and other sharp objects to avoid accidental injury.
- Operating procedures: Follow strict operating procedures, such as avoiding contact with the mouth when using a straw, avoiding eating or using personal items in the work area.
- Emergency plan : Develop and be familiar with emergency plans to deal with possible accidents, such as chemical leakage, fire or personal exposure.

- Health monitoring : Laboratory staff should conduct regular health checks, especially for potential pathogens.
- Waste decontamination : All potentially contaminated waste should be properly decontamination before leaving the laboratory.
- Record and report : maintain detailed experimental records, including all experimental procedures and any accident or exposure events.
- Safety identification : Set up obvious biological hazard identification at the entrance and exit of the laboratory to remind personnel to pay attention to safety.

5. Product use steps

5.1 Calculate the required volume of virus vector based on MOI and virus titer, and add the virus vector to the activated cell suspension;

5.2. Based on the total volume of the cell suspension after adding the virus vector, add Viral E-hancer A according to the volume ratio of Viral E-hancer A: cell suspension 1:30, and add Viral E-hancer B according to the volume ratio of Viral E-hancer B: cell suspension 1:100, slowly blow and mix well.

5.3. Cells, viral E-hancer, and virus mixture were centrifuged at room temperature for 30min at 1000g (increasing by 5 and decreasing by 5) for 30min. After centrifugation, they were placed in a 37 °C and 5% CO_2 incubator for further cultivation for 24 ± 2h;

5.4 Take out the transduced cell suspension, centrifuge at room temperature for 8 minutes at 400g, discard the supernatant, resuspend the cell pellet with complete culture medium, adjust the cell density to 5E5 cells/mL, and culture the cells in a 37 °C and 5% CO_2 incubator. Add supplement medium every 2-3 days to adjust the cell density to 5E5 cells/mL.

Note:

1. The dosage mentioned in this instruction is recommended, and users can explore the dosage gradient according to their own process conditions;
2. Centrifugal transduction steps is the recommended step, which users can selectively adopt according to their own process conditions.

6. Associated products

Product name	Product Code
Blood / tissue / cell genomic DNA extraction kit	HG-NA100
CAR / TCR gene copy number detection kit (qPCR- fluorescent probe method)	HG-CA001
RCL (VSVG) gene copy number detection kit (qPCR fluorescence probe method)	HG-RC001
Mycoplasma DNA Sample Preprocessing Kit (magnetic bead method)	HG-CL200
Mycoplasma DNA detection kit (qPCR-fluorescent probe method)	HG-ZY002
Mycoplasma DNA detection kit (qPCR-fluorescent probe method)	HG-ZY001
CRS Cytokine ELISA Detection Kit	HG-HC001

Product name	Product Code
Cell residual human interleukin 2 (IL-2) ELISA, test kit	HG-IL002
Cell residual human interleukin 7 (IL-7) ELISA, test kit	HG-IL007
Cell residual human interleukin 15 (IL-15) ELISA, test kit	HG-IL015
Cell residual human interleukin 21 (IL-21) ELISA, test kit	HG-IL021
Human interferon γ (IFN- γ) ELISA, test kit	HG-IF001
Viral E-hancer C（ROU）	HG-PTD001-C-R
Viral E-hancer C（GMP）	HG-PTD001-C-G
Viral E-hancer D	HG-PTD001-D
NK / TIL cell amplification reagent (GFP tag)	HG-FEC001-RG
NK cell amplification kit	HG-POC004
CD 19 CAR-T cell preparation kit	HG-POC001
Cell kill detection kit (suspended target cells)	HG-CKK001

7. Contact information

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This document has been reviewed and approved by the quality department.

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