User Manual

Travirtide

for Transduction Enhancement

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Please read the entire Manual before starting your Experiments!
Carefully note the handling and storage conditions.
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1 Introduction

This protocol outlines the procedure for viral transductions of target cells such as immune cells, e.g. the introduction of chimeric antigen receptors (CARs) in T-lymphocytes derived from leukapheresis or peripheral blood lymphocytes (PBMCs).

The efficacy and degree of enhancement might differ amongst different viral vectors, their envelope protein, respectively, and cell types.

1.1 Components

Travirtide
(RUO 0.5 mg, CGP 1mg)
Travirtide was chemically synthesized, purified and analyzed via LC-MS.

Required but not included:
Anhydrous, cell culture grade DMSO.

Optional:
DMSO-resistant sterile filter, e.g. Millex-LG Millipore Syringe filter with 0.2 µm PFTE membrane, 13 mm; Merck Millipore, product code #SLLG013SL

1.2 Storage & Handling

Optimal storage temperature for the freeze-dried Travirtide is -20°C or below, preferably -80°C.

We recommend to use freeze-dried Travirtide within 6 month upon receipt.

Once dissolved, the stock solution can be stored at -80°C to -20°C for two month *(Refer to section 4.2 for additional details).*

Avoid repeated thawing/freezing cycles of stored aliquots.

2 Experimental Protocols

Note: The following procedure is provided as a guideline only. The optimal experimental conditions will vary depending on the sample and instruments used. The optimal experimental conditions must be established by the user.

2.1 Reconstitution of Travirtide

1. Dissolve 0.5 mg lyophilized Travirtide in 50 µL DMSO (or 1 mg Travirtide in 100 µL DMSO) to obtain a stock solution with a concentration of 10 mg/mL. We recommend using fresh, anhydrous DMSO at cell culture grade. This stock solution can be stored at -20°C or colder for a maximum of 2 month.

2. OPTIONAL: Filter the resulting stock solution into a clean tube under aseptic conditions (e.g. class II laminar flow cabinet) using a DMSO-resistant filter (e.g. Millex-LG Millipore Syringe filter with 0.2 µm PFTE Membrane, 13 mm; Merck Millipore, Product Code *SLLG013SL*). Please note the hold-up volume of the filter, which is approximately 30 µL.

3. The stock solution is further diluted with PBS to obtain a 1 mg/mL working solution. After dilution, the solution is vortexed vigorously and left for 10-15 min at room temperature. You can dilute this working solution further with PBS depending on the experimental setup. For example: Dilute working solution to 100 µg/mL to obtain a recommended final concentration of 5 µg/mL at the time of transduction. Unused working solution must be discarded afterwards and should not be stored.

2.2 Transduction

We highly recommend to carefully titrate the amount of Travirtide depending on the viral vector, cell culture und expansion system used. As a starting point, Travirtide can be used at a final concentration of 5 µg/mL at the time of transduction.

For transduction, the working solution is gently added to the virus-containing supernatant or purified virus in a volume ratio of 1 to 1 and incubated for 10-15 minutes at room temperature. The cells are then inoculated with the virus/peptide mixture and incubated under standard conditions.

Example - Transduction protocol for a 24-well plate with γ-retrovirus.

1. Harvest the cells to be transduced and determine cell viability and cell number. For a 24 well plate, seed 500.000 cells in 360 µL medium per well. Please ensure a high viability of the cells.
2. Dilute the Travirtide working solution with PBS to e.g. 100 µg/mL and add 20 µL of this dilution to 20 µL of virus/viral supernatant. Incubate the mixture for 10 to 15 minutes at room temperature.
3. Add the 40 µL of virus-Travirtide mixture to the cells and place the whole plate in an incubator at 37°C, 5 % CO_2 and 95 %rH for 16 to 20 hours. (The Travirtide concentration is 5 µg/mL).
4. Add 1600 µL medium (including serum) to the cells and return the plate into the incubator.
5. 48 hours after transduction, the cells can be analyzed for the expression of viral genes like reporter gene GFP.

We strongly recommend optimizing MOIs, cell density and Travirtide concentration as well as the ratio of cells to virus-Travirtide mixture depending on the culture systems used. Travirtide can be used in the presence of serum, however transduction enhancement is higher under serum-reduced or serum-free conditions. In the latter case, serum should be added after transduction for the culture period.

3 Contact Us

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4 Product Use & Liability

THE PRODUCTS ARE FOR EXPERIMENTAL LABORATORY USE ONLY AND NOT INTENDED FOR HUMAN OR HOUSEHOLD USE.
Only qualified personnel should handle these chemicals.
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