



Innovative Peptide Solutions

Protocol

Protransduzin™ RUO

for Transduction Enhancement

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Product Use & Liability

THE PRODUCT IS FOR RESEARCH AND IN VITRO USE ONLY AND NOT INTENDED FOR HUMAN OR HOUSEHOLD USE.

Only qualified personnel should handle these chemicals.

Furthermore, JPT Peptide Technologies stresses that missing hazard warnings do not mean that the relevant product is harmless. In regard to classification the products are only for research purposes. JPT Peptide Technologies cannot be made responsible for damages arising from misuse of any product.

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1. Introduction

This protocol suggested is for viral transduction 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)

2. Components

Protransduzin™ contains approx. 2 mg peptide.

Protransduzin™ was chemically synthesized, purified and analyzed by LC-MS.

Required but not provided along with the product:

Cell culture grade anhydrous DMSO, e.g. Hybri-Max™; Sigma-Aldrich; Product code #D2650

Optional:

DMSO-resistant sterile filter device, e.g. Millex-LG Millipore Syringe filter with 0.2 µm PFTE Membrane, 13 mm; Merck Millipore, Product Code #SLLG013SL

3. Storage and Handling

Optimal storage temperature for the freeze-dried Protransduzin™ is -20°C.

Freeze-dried Protransduzin™ is recommended to be used within 12 month.

Once dissolved, stock solution can be stored at -20°C for two month (See 4.2).

Avoid repeated thawing/freezing cycles of stored aliquots.

4. Experimental Protocol

4.1 Reconstitution of the Freeze-Dried Protransduzin™

1. Dissolve 2 mg lyophilized Protransduzin™ (PTD) in 200 µL DMSO to yield a stock solution with a concentration of 10 mg/ml. Resuspension may be enhanced by sonification or warming PTD/DMSO solution to 37 °C. We recommend using fresh, anhydrous DMSO at cell culture grade.
2. OPTIONAL: Sterile-filter the obtained stock solution into a clean tube under aseptic conditions (e.g. class II laminar flow cabinet) using a DMSO-resistant filter (Millex-LG Millipore Syringe filter with 0.2 µm PTFE Membrane, 13 mm; Merck Millipore, Product Code *SLLG013SL*). Fill a 1 ml-Luer-outlet-syringe with the solution, remove the cover from the package that contains the sterilizing filter unit and attach the syringe to the Millex-LG unit. Place the filter assembly over the clean tube and push the plunger to deliver the filtered solution into the tube. The solution hold-up volume (loss) after air purge is <25 µl.
3. To activate fibril formation, dilute the reconstituted PTD stock solution with PBS to yield a working solution of 1 mg/ml. After 10 min the fibril formation should be completed.
4. Use working solution immediately. Working solution is stable for 12 hours. Unused working should be discarded afterwards.

4.2 Transduction using Protransduzin™

We recommend to carefully titrate the amount of Protransduzin™ depending on the used viral vector and culture und expansion system. As a starting point you can use Protransduzin™ at a final concentration of 10-100 µg/mL.

For transduction gently add working solution to virus containing supernatant or purified virus to a final concentration of 10 -100 µg Protransduzin™ (please optimize) and the multiplicities of infection (MOI) of choice; incubate for 5 mins at room

temperature. Inoculate cells at density of 5×10^5 cells/mL with the virus/PTD mixture and incubate under standard conditions.

NOTE: We highly recommend optimizing MOIs, cell density and Protransduzin concentration and the ratio of cells and virus/PTD-supernatant depending on the used culture systems. Protransduzin™ can be used in the presence of serum but transduction enhancement will be higher under reduced serum or serum-free conditions.

4.3 Concentration of Virions using Protransduzin™

Dilute the PTD stock solution 10-fold with PBS or medium (w/o serum) to 1 mg/ml (PTD working solution). Vortex for 2 seconds. Add freshly prepared PTD working solution to the virus stock to obtain a concentration of 10µg/ml PTD. Resuspend PTD-A/virus solution twice (5 µg/mL PTD final concentration). Incubate 5 min at room temperature. Centrifuge PTD/virus solution at 10 000 g for 5 min at room temperature. Carefully remove supernatant using a pipette, and resuspend the PTD /virus pellet in 1/10 of the original volume using a medium or buffer of choice. Perform titer determination or use concentrated virus directly for transduction. If performing transduction with concentrated virus final concentration of PTD should not exceed 50µg/mL.

5. Related Products

Protransduzin™ Clinical Grade

This product has been produced in an enhanced manufacturing environment to be used as starting material for the production of *ex vivo* cell therapies.

PepTrack™ Peptide Libraries

Our flexible customized peptide libraries are optimized for antigen-specific stimulation in T-cell assays, immune monitoring, T-cell epitope identification, and development of cellular therapy approaches.

Clinical Grade Peptides & Pools

The Clinical Grade production is regulated beyond ISO 9001:20015 guidelines for the more stringent product requirements of immunotherapy as well as vaccine & drug development.

MHC Multimers

MHC Multimers detect the presence of antigen-specific T cells for robust immune monitoring.