Protransduzin®-A

INTENDED USE

Protransduzin®-A is a highly efficient transduction enhancer to increase retro- and lentiviral gene transfer into adherent and suspension cells. Moreover, Protransduzin®-A allows a rapid and convenient concentration of viral particles by brief low speed centrifugation in a standard bench top centrifuge which also allows to resuspend virions in the medium of choice.

INTRODUCTION

Low transduction efficiencies of retro- and lentiviral vectors represent a significant problem in basic and applied research. Protransduzin®-A is a 12-mer peptide that assembles into nanofibrils which efficiently and rapidly associate with viral particles[1] . These virus-loaded fibrils bind to cellular membranes thereby increasing viral attachment, and consequently fusion and transduction. Protransduzin®-A enhances not only transduction efficiencies of retro- and lentiviral vectors that are currently used for gene transfer and therapy, but also of replication-competent lentiviruses such as HIV and SIV, or γ-retroviruses such as MLV. Notably, this transduction/infection promoting effect is independent of the viral glycoprotein and works for all routinely used pseudotypes. Protransduzin®-A nanofibrils enhances retro- and lentiviral gene delivery into all susceptible cell types, including T cells, macrophages and hematopoietic stem cells[1], and is also substantially more effective than other reagents commonly used to increase transduction (fig. 1)[1,2].

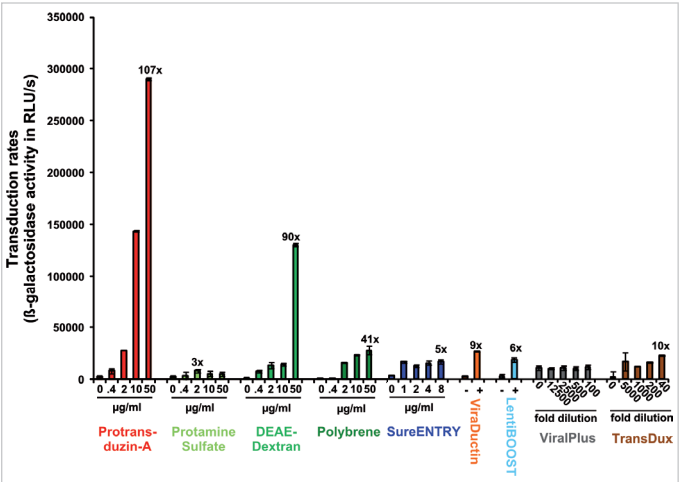

Figure 1: Protransduzin®-A is a highly efficient transduction enhancer. Comparison of Protransduzin®-A with other commercially available transduction enhancers for a non-coating procedure. Lentiviral particles were treated with the indicated concentrations of Protransduzin®-A or Protamine sulfate, DEAE-dextran and Polybrene (all from Sigma Aldrich), and then used to inoculate TZM-bl cells. The remaining transduction enhancers were used as recommended by the manufacturer (SureENTRY: Qiagen; ViraDuctin: Cell Biolabs; LentiBOOST: Sirion Biotech; ViralPlus: Applied Biological Materials (ABM) Inc; TransDux: System Biosciences). Infection/transduction rates were determined 3 days later by quantifying ß-galactosidase activities in cellular lysates (n=3, mean ± SD). The numbers above the columns give the maximum enhancement of transduction relative to the control containing no additive.

Studies in mice demonstrated comparable transduction and engraftment rates of bone marrow cells after application of a one-step Protransduzin®-A -based protocol compared to a time-consuming conventional multi-step spin-infection protocol using RetroNectinTM that requires coating[1]. In addition, the formation of Protransduzin®-A/virus complexes allows for the concentration of virions by brief low-speed centrifugation making the use of time-consuming ultra-centrifugation dispensable. Thus, Protransduzin®-A represents a flexible, highly effective, convenient, and affordable new tool to facilitate work with infectious retroviruses and/or retrovirus based vectors in basic and clinical research.

PRINCIPLE

Protransduzin®-A sequesters virions from the surrounding medium. The formation of these macroscopic complexes increases the number of viral particles at the cell surface. This scenario might emerge from neutralization of the repulsion between the negatively charged viral and cellular membranes in conjunction with attractive interactions of excess positive charges on the fibrils with the cell surface. This ultimately results in enhanced fusion of the viral and cellular membranes.

MATERIAL SUPPLIED

2 mg / 10 mg lyophilized Protransduzin®-A peptide.

MATERIAL REQUIRED BUT NOT SUPPLIED

Dimethylsulfoxid (DMSO)

STORAGE AND STABILITY OF REAGENTS

The lyophilized peptide can be stored for 1 year at -20 °C. When dissolved in DMSO the peptide can be stored at -20 °C for 1 week. Use immediately after further dilution.

PROCEDURE

Perform all of the following steps under sterile conditions.

A. Protransduzin®-A (PTD-A) to increase retro- and lentiviral transduction efficiency

The following protocol works for adherent as well as for suspension cells.

1.	Dissolve PTD-A peptide in 200 µl DMSO to a concentration of 10 mg/ml (PTD-A stock solution).
2.	Dilute the PTD-A stock solution 10-fold with PBS or medium (w/o FCS) to 1 mg/ml (PTD-A working solution). Vortex for 2 seconds.
3.	Add freshly prepared PTD-A working solution to the virus stock of interest to obtain a concentration of 10–50 µg/ml PTD-A and resuspend twice.
4.	Incubate 5 min at room temperature.
5.	Inoculate cells with the PTD-A/virus mixture.
6.	Cultivate cells under standard conditions. Optionally, cell culture medium can be exchanged after 4 hours of incubation.

Note: For more details, see reference 1. Usually it is sufficient if the PTD-A/virus solution makes up ~10 to 20 % of the final cell culture volume to reach maximum transduction efficiencies. Cells may also be exposed to 100 % of the PTD-A/virus solution if required. If viral stocks contain used-up medium or have a low titer, PTD-A also permits concentrating virions in the medium of choice (see B)

B. Protransduzin®-A (PTD-A) to concentrate retro- and lentiviral vectors

1.	Dissolve PTD-A peptide in 200 µl DMSO to a concentration of 10 mg/ml (PTD-A stock solution).
2.	Dilute the PTD-A stock solution 10-fold with PBS or medium (w/o FCS) to 1 mg/ml (PTD-A working solution). Vortex for 2 seconds.
3.	Add freshly prepared PTD-A working solution to the virus stock to obtain a concentration of 10 µg/ml PTD-A.
4.	Resuspend PTD-A/virus solution twice.
5.	Incubate 5 min at room temperature.
6.	Centrifuge PTD-A/virus solution at 5 000–10 000 *g* for 5 min at room temperature.
7.	Carefully remove supernatant using a pipette, and resuspend the PTD-A/virus pellet in 1/10 of the original volume using the medium or buffer of choice.
8.	Inoculate cells with the concentrated PTD-A/virus mixture. Final concentrations of PTD-A in cell culture should not exceed 50 µg/ml.

Note: For more details, see reference 1.

PRECAUTIONS

- All reagents in the kit package are for *in vitro* use only.
- Work should be performed by skilled persons considering GLP (good laboratory practice) guidelines.

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TECHNICAL HINTS

- Do not interchange different lot numbers within the same assay.
- Control samples should be analyzed with each experiment.
- Reagents should not be used beyond the expiration date stated on the label.
- The experiment should always be performed according the enclosed manual.

GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

REFERENCES

1. Yolamanova M, Meier C, Shaytan AK, Vas V, Bertoncini CW, Arnold F, Zirafi O, Usmani SM, Müller JA, Sauter D, Goffinet C, Palesch D, Walther P, Roan NR, Geiger H, Lunov O, Simmet T, Bohne J, Schrezenmeier H, Schwarz K, Ständker L, Forssmann WG, Salvatella X, Khalatur PG, Khokhlov AR, Knowles TP, Weil T, Kirchhoff F, Münch J. Peptide nanofibrils boost retroviral gene transfer and provide a rapid means for concentrating viruses. Nat Nanotechnol. 2013 Feb;8(2):130-6. doi: 10.1038/nnano.2012.248. Epub 2013 Jan 20.
2. Meier C, Weil T, Kirchhoff F, Münch J. Peptide nanofibrils as enhancers of retroviral gene transfer. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2014 Sep;6(5):438-51. doi: 10.1002/wnan.1275. Epub 2014 May 28.

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