Miltenyi Biotec

Vectofusin-1®

Order no. 130-111-163

Contents

1. Description

This product is for research use only.

Components	1 mg Vectofusin-1®: polypeptide
Capacity	For the preparation of up to 100 mL transduction medium.
Product format	Lyophilized peptide containing stabilizer.
Purity	≥92% (HPLC)
Storage	Store lyophilized product at –20 °C. The expiration date is indicated on the vial label. Upon reconstitution aliquots should be stored at –70 °C or below. Avoid repeated freeze-thaw cycles.

This product contains no preservative and is sterile filtered; always handle under aseptic conditions.

1.1 Background information

Gene transfer into suspension cells such as T cells or hematopoietic stem cells using retroviral vectors has several therapeutic applications ranging from monogenic diseases, infectious diseases, and cancer. Viral transduction of suspension cells using retroviral vectors is often inefficient and requires the use of transduction enhancers. Vectofusin-1® is a fully synthetic non-toxic cationic amphipathic peptide with viral transduction enhancing capacity, enabling higher transduction levels with low amounts of retroviral vector. Vectofusin-1 promotes the entry of several retroviral pseudo-types into target cells when added to the culture medium.

1.2 Applications

- Enhancement of viral transduction of suspension cells.

2. Protocol

2.1 Cell preparation

For transduction of immortalized cell lines, seed the cells to the desired cell concentration for the transduction step. For transduction of primary cells, cultivate the cells for 1–3 days in a suitable activation medium to make the cells susceptible to genetic modification with retroviral vectors, e.g., for human T cells, cultivate cells in TexMACS™ Medium containing T Cell TransAct™, human and cytokines; for human hematopoietic stem cells cultivate the cells in StemMACS™ HSC Expansion Medium containing StemMACS HSC Expansion Cocktail, human or other suitable cytokines.

2.2 Reagent requirements

- Culture medium for transduction without serum or lipids, e.g., IMDM or RPMI.
- (Optional) TexMACS Medium, research grade (# 130-097-196)
- (Optional) T Cell TransAct, human (# 130-111-160)
- (Optional) StemMACS HSC Expansion Media XF, human (# 130-100-473)
- (Optional) StemMACS HSC Expansion Cocktail, human (# 130-100-843)

2.3 Recommendations for reconstitution

1. For reconstitution of the lyophilized peptide take the vial from –20 °C and warm-up to room temperature.
   ▲ **Note:** Do not open the vial by removing the rubber plug.
2. To dissolve the Vectofusin-1 fill a sterile syringe (2 mL) with 1000 µL of sterile water.
3. Slowly inject the water with a sterile needle through the center of the rubber plug into the vial containing the lyophilized Vectofusin-1.
4. Vortex the solution to completely dissolve the lyophilized peptide.
   The concentration of the stock solution of Vectofusin-1 is 1 mg per mL.
5. Remove the rubber plug and aspirate the stock solution with a pipette.
6. To avoid repeated freeze-thaw cycles prepare working aliquots from the stock solution.
7. Store the working aliquots at –70 °C.

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 877 591 1060
macs@miltenyibiotec.com

140-005-131.02

2.4 Transduction

▲ Before transduction, determine the cell concentration to be able to determine the amount of viral vector required to reach the desired multiplicity of infection (MOI).

1. Prepare cells in the desired format and cell concentration.

   ▲ **Note:** The volume should be low enough to allow the addition of the viral vector supernatant, Vectofusin-1, and plain culture medium without serum or lipids. For calculation examples refer to "Preparation examples of Vectofusin-1 transduction mixtures" below.

2. Thaw aliquot of Vectofusin-1 at room temperature. Vortex thoroughly before use.

3. The required final concentration of Vectofusin-1 for transduction is 10 µg/mL in the total culture volume.

4. Dilute viral vector or viral supernatant and Vectofusin-1® separately by adding plain culture medium. Both diluted solutions shall have identical final volumes.

   ▲ **Note:** For calculation examples refer to "Preparation examples of Vectofusin-1 transduction mixtures" below.

5. Mix diluted viral vector or viral supernatant and diluted Vectofusin-1 solutions and vortex or flick the mixture.

6. Add the mixture of viral vector or viral supernatant and Vectofusin-1 to the cell suspension and pipette up and down.

   ▲ **Note:** Duration between mixing the viral vector and Vectofusin-1 and addition of the mixture to the target cells should not exceed 10 minutes.

7. Incubate at 37 °C.

8. (Optional) To reach a higher transduction performance, centrifuge cell samples at 400×g for 1–2 hours at 32 °C followed by static incubation at 37 °C. Wash cells once 6–24 hours after transduction with transduction medium.

9. Cultivate cells at 37 °C.

Preparation examples of Vectofusin-1 transduction mixtures

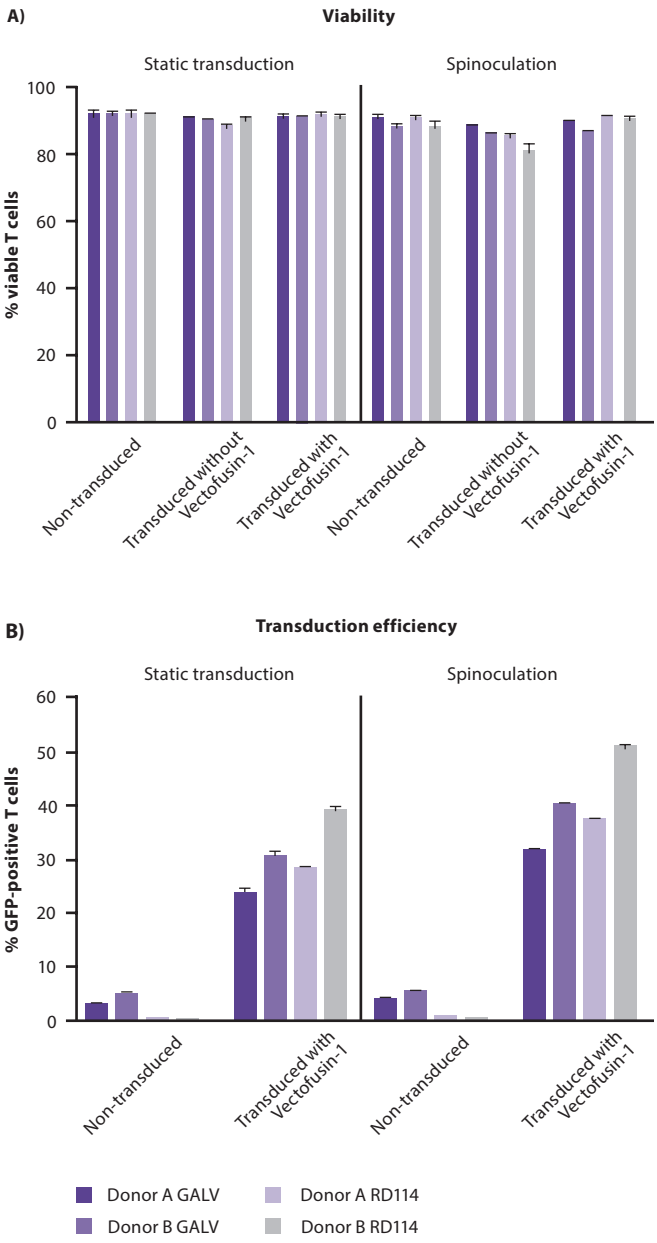
Vectofusin-1 can be used with both purified viral vector and cell culture supernatants. Examples for preparing transduction solutions with different volumes of viral vector or viral supernatant and in different cell culture formats are given below.

	96 well (µL)	24 well (µL)	CliniMACS Prodigy (mL)
Retroviral supernatant	20	–	15
Purified vector	–	5	–
Medium	30	245	–
Diluted viral vector solution	**50**	**250**	**15**
Vectofusin-1 (1000 µg/mL)	2	20	1
Medium	48	230	14
Diluted Vectofusin-1 solution	**50**	**250**	**15**
Cell volume already in culture	100	1500	50 + 20*
Total culture volume after addition of viral vector and Vectofusin-1	**200**	**2000**	**100**

*Extra medium supplied during program activity "Transduction" of the CliniMACS Prodigy T Cell Transduction Process (TCT Process).

3. Examples

Human CD4$^+$ and CD8$^+$ T cells were magnetically enriched with CD4 and CD8 MicroBeads and were activated with T Cell TransAct™ in TexMACS™ Medium supplemented with IL-2. Two days after activation, T cells were transduced with gamma-retroviral vectors encoding GFP in the presence of Vectofusin-1®. Vectofusin-1 was added at a final concentration of 10 µg/mL to the cell culture medium. Transduction was performed either at 37 °C in an incubator (static) or the T cells were centrifuged at 400×g for 2 hours at 32 °C (spin) before transfer to 37 °C. T cell viability was assessed by flow cytometry after staining T cells with Viobility™ 405/452 Fixable Dye (A). Transduction medium was exchanged either 6 hours or 24 hours after transduction and GFP expression was analyzed on day 7 of cultivation using a MACSQuant® Analyzer (B).



140-005-131.02

4. References

1. Fenard, D. *et al.* (2013) Vectofusin-1, a new viral entry enhancer, strongly promotes lentiviral transduction of human hematopoietic stem cells. Mol Ther Nucleic Acids 2: e90.
2. Ingrao, D. *et al.* (2014) Concurrent measures of fusion and transduction efficiency of primary $CD34^+$ cells with human immunodeficiency virus 1-based lentiviral vectors reveal different effects of transduction enhancers. Hum Gene Ther Methods 25(1): 48–56.
3. Majdoul, S. *et al.* (2016) Molecular determinants of Vectofusin-1 and its derivatives for the enhancement of lentivirally mediated gene transfer into hematopoietic stem/progenitor cells. J. Biol. Chem. 291(5): 2161-2169.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.