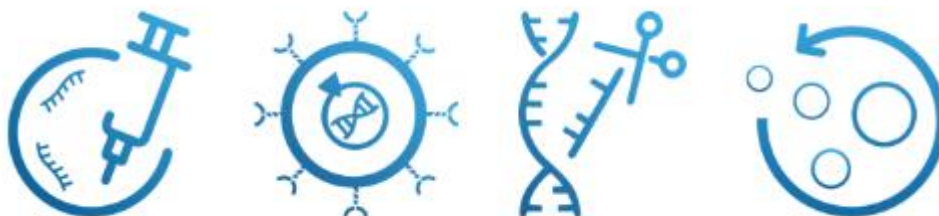




LentiFlash®: A non-viral biological RNA-delivery system

<https://www.flashtherapeutics.com/technology/lentiflash-technology/>



LentiFlash®

Catalog Number referring to this User Manual:

0057VCT; 0059VCT

Contents:

- Cat.# 0057VCT: 2x20µl of RLP-CRE-ZsGreen1-11 lentiviral particles.
 - Cat.# 0059VCT: 2x20µl of RLP-CRE-11 lentiviral particles.
- Please refer to the certificate of Analysis (CoA) for the titer of your particular lot.

Description:

LentiFlash® particles product is a non-viral biological RNA-delivery system based on a chimeric lentiviral vectors for transient RNA transfer. This RNA packaging system is based on bacteriophage MS2-Coat-lentiviral chimeras. It has been constructed by exploiting bacteriophage MS2-Coat and its cognate 19-nt stem loop instead of the natural lentiviral Psi packaging sequence to manage RNA packaging into the lentiviral particle.

The resulting engineered chimeric particles promote effective packaging of RNAs and enable efficient transfer of biologically active RNAs in various cell types including immortalized, primary and stem cells (*Prel et al., Highly efficient in vitro and in vivo delivery of functional RNAs using new versatile MS2-chimeric retrovirus-like particles, Molecular Therapy - Methods & Clinical Development 2015*). LentiFlash® particles are VSV-G pseudotyped lentiviral particles carrying non-viral mRNA or other types of RNAs [produced by co-transfection of producer cells] for delivering these RNAs into mammalian cells.

Our LentiFlash® particles deliver mature mRNA that leads directly to transient protein expression. There is no waiting for promoter-driven translation and there is no genomic integration. These VSV-G pseudotyped particles are capable of transferring RNA into dividing and non-dividing cells. The high purification level of these LentiFlash® particles allows transduction of primary cells and immortalized cell lines.

Handling and Storage:

Store at -80°C. Keep frozen until use. Avoid repeated freezing and thawing. The use of gloves and disposable lab coats while working with LentiFlash® particles is strongly recommended. This product must only be handled in a biosafety cabinet under BSL-2 conditions. LentiFlash® particles are stable for at least 1 year after receipt when stored at -80°C. **After thawing, immediately place on ice** (please refer to the thawing protocol included in this document). The LentiFlash particles are packaged in working aliquots and can be thawed just before use. In case a second freeze-thaw cycle is required for your application, expect a decrease of about 30% in viral vector titer after the second freeze-thaw cycle. This product is distributed for research use only. It is not for use in diagnostic procedures as the safety and efficacy of this product in diagnostic or other clinical uses has not been established.

Safety precautions:

The greatest safety risk associated with viral delivery systems comes from the potential generation of recombinant viruses that are capable of autonomous replication during the packaging process. The Flash Therapeutics LentiFlash® platform eliminates these hazards by combining a non-viral RNA encapsidation with a unique manufacturing process. As for classical lentiviral systems, the viral genes that facilitate the enclosing of the sequence in a viral capsid (e.g., Gag, Pol, Env) are distributed on multiple helper plasmids (which do not contain significant regions of homology) during packaging. This strategy further prevents the probability of recombination events that might otherwise generate viruses capable of autonomous replication. With these safety measures, the Flash Therapeutics LentiFlash® particles can be used in standard Biosafety Level 2 tissue culture facilities as they are used to deliver RNA into cells in culture as a transfection reagent.

Directions for use, thawing protocol:

The LentiFlash® particles should be taken out of the -80°C freezer and placed on ice immediately prior to use. Thaw the LentiFlash® particles on ice. Once thawed, the LentiFlash® particles should be used for transduction as soon as possible to avoid degradation.

- 1- Just before transduction, remove the tubes of LentiFlash® supernatant from the -80°C freezer and thaw them on ice.
- 2- It is essential to avoid thermal shock to the cells and particles. If the LentiFlash® particles are diluted in medium, use a medium that has been brought to room temperature in order to minimize the heat shock to the particles and the cells.
- 3- Five minutes before transduction, remove the tubes from ice and allow warming to room temperature.

In case a second freeze-thaw cycle is required for your application, expect a decrease of about 30% in LentiFlash® particles titer after the second freeze-thaw cycle. Do not freeze LentiFlash after the second thawing.

Directions for use, Materials Required but Not Provided:

1. 6-well plates (TC grade)
2. Cell counter / hemocytometer
3. Complete media FBS supplemented
4. Phosphate Buffered Saline (PBS)
5. Polybrene® (Hexadimethrine bromide – Sigma: 107689-10G)

Directions for use, Protocol for Cells Transduction:

Day 0 : Cells seeding

For the transduction of immortalized cell lines, seed the cells by plating from 7×10^3 to 3×10^6 cells per well according to the table below (25000 cells/cm^2) to reach 50% of cells confluency on transduction day. For primary cells, seed the cells according to your usual seeding conditions. Use the same culture medium that is used to maintain target cells in a proliferative state. Incubate overnight in a 37°C, 5% CO₂ incubator. Be sure to include a control well for counting the number of cells on the day of transduction.

Plastic support	96 well plate	24 well plate	6 well plate	T25 flask	T75 flask	T150 flask
Cells / well	8×10^3 cells	50×10^3 cells	250×10^3 cells	625×10^3 cells	$1,88 \times 10^6$ cells	$3,75 \times 10^6$ cells

Day 1 : Cells transduction

- A) Count the number of cells in the control well to determine the amount of LentiFlash® particles needed to achieve the target PP/cell dose (Physical Particle/cell) and to keep this dose constant from one experiment to another.
- B) Using the following equation, determine the volume of each LentiFlash® batch required to achieve the PP/cell dose of your choice. Please pay attention to the titer as it may vary by lot:

$\text{Lenti-Flash volume required } (\mu\text{l}) = \frac{\text{Number of cells counted}}{\text{Viral Vectors Titer (PP/mL)}} \times \text{Dose PP/cell} \times 1000$
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Example: LF-MS2-CRE vector at $1E^{12}$ PP/ml. Transduction of cells at PP/cell dose = 100 000 in a 24 wells plate:

- Determination of particles quantity needed for 1 well:

$50\ 000\ \text{cells} \times 100\ 000\ \text{PP/cell} = 5 \times 10^9\ \text{PP/ well}$

- Determination of viral vector per well:

$5 \times 10^9\ \text{PP} / 1 \times 10^{12}\ \text{PP/ml} = 0,005\ \text{ml}$, either $5\ \mu\text{l}$ viral vector per well.

- Preparation of transduction mix:

$5\ \mu\text{l}$ of viral vector

$5\ \mu\text{l}$ of Polybrene at $800\ \mu\text{g/ml}$ (final concentration = $4\ \mu\text{g/ml}$)

$990\ \mu\text{l}$ completed culture media

- C) Thaw the required amount of LentiFlash® according to the thawing protocol provided above. Homogenize by pipeting up and down and not by inverting the vial upside down.
- D) Prepare the transduction mix by adding the required volume of thawed LentiFlash® to complete media containing Polybrene® (800µg/ml) as described in the table below:

Plastic support	96 well plate	24 well plate	6 well plate	T25 flask	T75 flask	T150 flask
LentiFlash® + completed media volume	199µl	995 µl	3,98 ml	4,975ml	9,95ml	19,9ml
Polybrene (800µg/ml)	1 µl	5 µl	20 µl	25µl	50 µl	100 µl
Final Volume	200µl	1ml	4ml	5ml	10ml	20ml

- E) Discard the medium from each well and add the transduction mix to the cells (be careful to apply the transduction mix to the well edges to avoid any cell disruption). Gently rock the plate from side to side to mix LentiFlash® particles onto target cells.
- F) Incubate cells with transduction mix for at least 4h (maximum 16h) in a 37°C, 5% CO₂ incubator.
- G) After incubation, discard the transduction mix and wash the cells once with PBS. Then, discard the PBS and replace it with cell culture media.
Caution: discarded medium may contain residual functional particles. Discarded medium are classic waste that must be treated like other BSL-2 laboratory waste.

- H) Incubate cells in a 37°C, 5% CO₂ incubator for at least 4h before characterization of transgene expression.

If necessary, perform a second transduction by repeating the steps described above. A second transduction must not be performed before 48h after the first transduction.

Notice to purchaser:

Purchaser represents and warrants that it will use the Flash Therapeutics LentiFlash® particles for research purposes only: not for diagnostic use, not for resale, and not for use in humans or veterinary applications. Flash Therapeutics will not be held responsible for patent infringement or other violations that may occur with the use of their products. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply.