



PEG-*it*[™] Virus Precipitation Solution (5×)

Cat. # LV810A-1/ LV825A-1

User Manual

A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the Licensing and Warranty Statement contained in this user manual.

Store kit at 4°C on receipt

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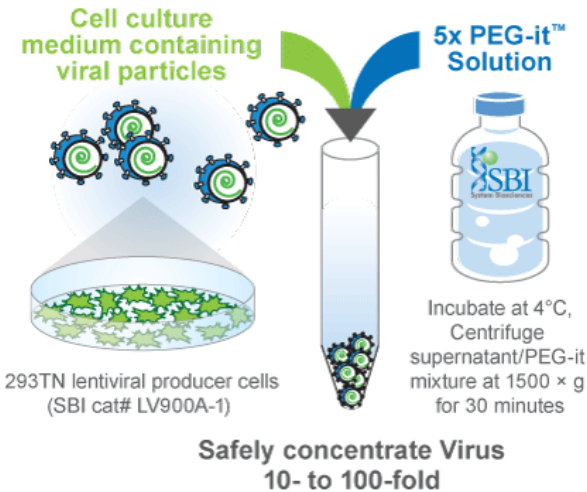
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List of Components

Each PEG-*it*[™] bottle provides enough material to precipitate virus from viral supernatant according to the table below:

Volume PEG-it	Catalog Number	Viral Supernatant
100 ml	LV810A-1	400 ml
250 ml	LV825A-1	1000 ml

The kits are shipped at room temperature or on blue ice and should be stored at +4°C upon receipt. Properly stored kits are stable for 1 year from the date received.



I. Protocol

A. Protocol

Lentiviral Vector Concentration by PEG-*it*TM Precipitation

PEG-*it*TM Virus Precipitation Solution provides a simple and highly effective means to concentrate lentiviral particles. PEG-*it* is a formulation of polyethylene glycol optimized for the precipitation of all lentiviral-based particles. **The PEG-*it* Virus Precipitation Solution is a 5x solution.**

1. Transfer supernatant to a sterile vessel and add 1 volume of cold PEG-*it* Virus Precipitation Solution (4°C) to every 4 volumes of Lentivector-containing supernatant. (*Example: 5ml PEG-*it* with 20ml viral supernatant*).
2. Refrigerate overnight (at least 12 hours). Lentivector-containing supernatants mixed with PEG-*it* Virus Precipitation Solution are stable for up to 4-5 days at 4°C.
3. Centrifuge supernatant/PEG-*it* mixture at 1500 × *g* for 30 minutes at 4°C. After centrifugation, the Lentivector particles may appear as a beige or white pellet at the bottom of the vessel.
4. Transfer supernatant to a fresh tube. Spin down residual PEG-*it* solution by centrifugation at 1500 × *g* for 5 minutes. Remove all traces of fluid by aspiration, taking great care not to disturb the precipitated Lentiviral particles in pellet.
5. Resuspend/ combine lentiviral pellets in 1/10 to 1/100 of original volume using cold, sterile Phosphate Buffered Saline (PBS) or DMEM containing 25mM HEPES buffer at 4°C.
6. Aliquot into cryogenic vials and store at -70°C until ready for use.

Precipitation of Lentivector particles from large volumes can be achieved by using the Corning 250 mL polypropylene centrifuge tube (Cat. # 430776), following manufacturer's instructions.

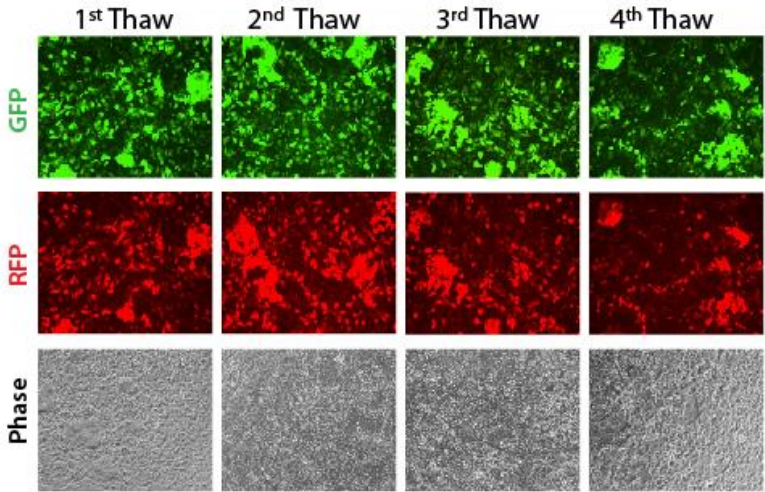
For more information on the viral production or transduction processes, please see SBI's [Lentivector Expression Systems: Guide to Packaging and Transduction of Target Cells](#).

B. PEG-it Protects Concentrated Virus

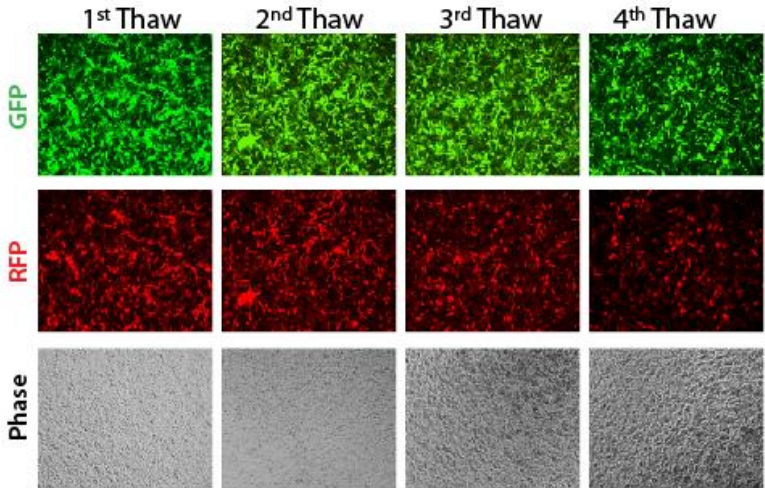
Not only does the PEG-it virus concentrate your lentivirus preparations by over 100-fold, it also acts as a cryopreservative agent. Lentivirus concentrated with PEG-it lasts longer in the freezer and also survives quite well in aliquot freeze/thaw cycles. To test this, 50,000 HepG2 or HT1080 cells were seeded in 500 microliters of DMEM media containing 10% FBS in 24 well plate formats. The next day, the media was replaced with fresh media containing 1x TransDux virus transduction reagent (Catalog # LV850A-1, System Biosciences). Previously made frozen aliquots of packaged virus with a MSCV-GFP-T2A-RFP expression cassette (catalog# LV605VA-1) were thawed on ice and 2 ul of virus was added to each well in triplicate. The aliquots were immediately frozen in dry ice and thawed again on regular ice and 2 ul of virus was again added to another three wells. This process was repeated three more times. Seventy two hour post transductions, cells were imaged for green fluorescence and documented. The images below show how well PEG-it protected the virus from multiple freeze/thaw cycles and produced high levels of GFP and RFP virus transgene expression.



HepG2 Cells transduced with LV605VA-1 after several freeze-thaw cycles

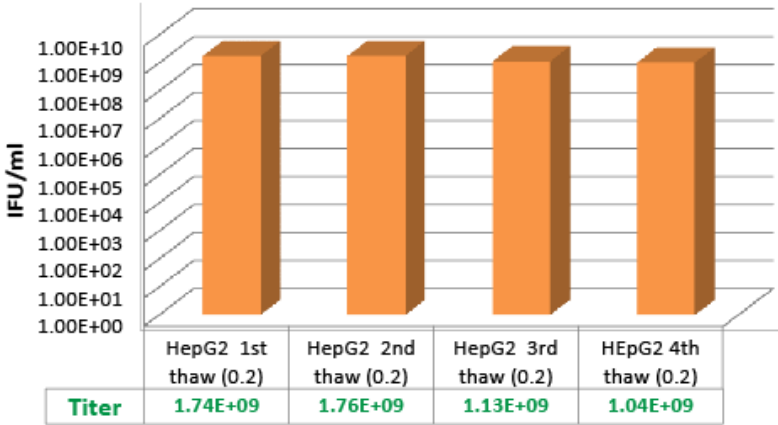


HT1080 Cells transduced with LV605VA-1 after several freeze-thaw cycles

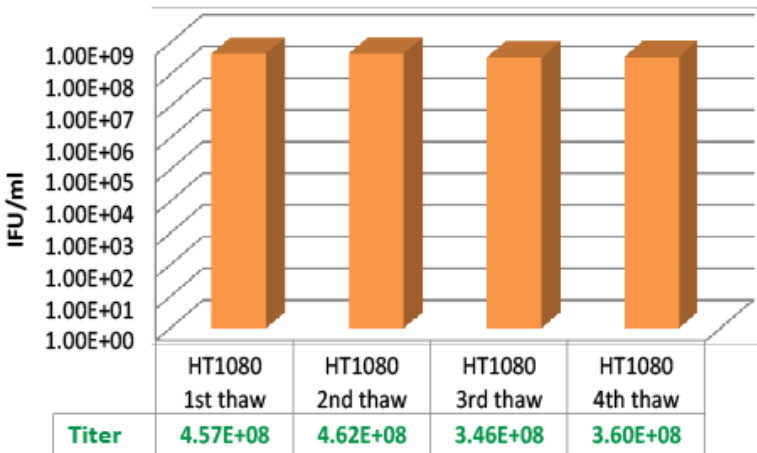


The cells were then lysed and viral infectious unit (IFUs) were measured using Global UltraRapid[™] Lentiviral Titer Kit (Catalog #LV961A-1, System Biosciences) following the kit's protocol. The Global UltraRapid[™] Lentiviral Titer Kit quantitates the number of host genome integrations reflecting the amount of active virus in the preparations. No significant loss of infectivities were measured during the virus freeze/thaw transduction tests.

HEPG2



HT1080



C. Related Products

- **Lentivector Packaging Kits**

For FIV-based Vectors: pPACKF1™ (Cat. # LV100A-1)

For HIV-based Vectors: pPACKH1™ (Cat. # LV500A-1)

Unique plasmid mixes that produce all the necessary viral proteins and the VSV-G envelope glycoprotein from vesicular stomatitis virus required to make active pseudoviral particles. Producer Cell Line 293TN (SBI Cat. # LV900A-1) transiently transfected with the packaging plasmids and an HIV-based lentiviral construct produce packaged viral particles containing the lentiviral construct of interest.

- **293TN Human Kidney Producer Cell Line** (Cat. # LV900A-1) For packaging of plasmid lentivector constructs.

- **UltraRapid Lentiviral Global Titering Kit** (Cat. # LV961A-1)

Allows you to accurately measure copy number (MOI) of integrated lentiviral constructs in genomic DNA of target cells after transduction with any of SBI's FIV or HIV-based lentivectors using sensitive qPCR.

- **Cloning and Expression Lentivectors** (many available)

Choose from FIV and HIV-based shRNA, cDNA, or TRE cloning and expression vectors. For a list of currently available vectors, please visit our website at www.systembio.com.

D. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

<http://www.systembio.com>

For additional information or technical assistance, please call or email us at:

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II. Licensing and Warranty Statement

Limited Use License

Use of the PEG-*it*TM Virus Precipitation Solution (*i.e.*, the “Product”) is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.

The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

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Limited Warranty

SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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