

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

Cat. # LV810A-1

Cat. # LV825A-1

User Manual

Store kit at 4°C on receipt

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.

(ver. 09-10-08)

Cat. # LV810A-1 Cat. # LV825A-1

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List of Components

Each PEG-*it*[™] bottle provides enough material to precipitate virus from 400 ml (cat# LV810A-1) or 1 liter (cat# LV825A-1) of viral supernatant.

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    100 ml PEG-it™ Virus Precipitation Solution (cat# LV810A-1)
    250 ml PEG-it™ Virus Precipitation Solution (cat# LV825A-1)
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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™ Precipitation

Generate lentiviral vector (Lentivector) particles in 293TN (SBI, Cat. # LV900A-1) or other 293 producer cell lines as described in SBI's user manual, Lentivector Expression Systems: Guide to Packaging and Transduction of Target Cells (see http://www.systembio.com). After collection of Lentivector-containing supernatant, the following protocol allows for precipitation and concentration of Lentivector particles, with a 1 to 2 log increase in final titer.

- 1. Collect supernatant and centrifuge at $3000 \times g$ for 15 minutes to remove cells and cell debris. Supernatant may be filtered through a $0.45~\mu m$ PVDF filter to further eliminate cellular debris. Please note that filtration may decrease the amount of virus in the supernatant, and should be reserved for clarifying supernatants that will be used to transduce target cells that are sensitive to cell debris.
- Transfer supernatant to a sterile vessel and add 1 volume of cold PEG-it Virus Precipitation Solution at 4°C to every 4 volumes of Lentivector-containing supernatant. The PEG-it Virus Precipitation Solution is a 5× solution. (Example: 5ml PEG-it with 20ml viral supernatant).
- 3. Refrigerate overnight (at least 12 hours). Lentivector-containing supernatants mixed with PEG-*it* Virus Precipitation Solution are stable for up to 2 days at 4°C.
- 4. 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.
- 5. Aspirate supernatant. 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.
- Resuspend Lentiviral pellet 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.
- 7. Aliquot in 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 manufacturers instructions.

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B. 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.
- Lentivector Rapid Titer PCR Kit (Cat. # LV950A-1 [for human cells], LV951A-1 [for mouse cells])
 Allows you to 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.
- Cloning and Expression Lentivectors (many)
 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.

C. 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:

System Biosciences (SBI) 1616 North Shoreline Blvd. Mountain View, CA 94043

Phone: (650) 968-2200

(888) 266-5066 (Toll Free)

Fax: (650) 968-2277

E-mail:

General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

II. Licensing and Warranty Statement

Limited Use License

Use of the PEG-*it*[™] 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.

Purchase of the product does not grant any rights or license for use other than those explicitly listed in this Licensing and Warranty Statement. Use of the Product for any use other than described expressly herein may be covered by patents or subject to rights other than those mentioned. SBI disclaims any and all responsibility for injury or damage which may be caused by the failure of the buyer or any other person to use the Product in accordance with the terms and conditions outlined herein.

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.

SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.

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