



LentiSuite Deluxe Kit

Cat# LV350A-1

User Manual

Please refer to PAC for storage temperatures

Version 2
10/16/2017

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Product Description

SBI's LentiSuite Deluxe Kit (Cat #LV350A-1) is an all-in-one kit to generate your own lentivirus from 3rd generation lentiviral vectors. The kit contains all of the reagents necessary to produce pseudoviral particles from 10 x 100mm plates or 4 x 150mm plates. The combination of the reagents below has been proven to produce high-titer pseudoviral particles for efficient transduction of most mammalian cells *in vitro*, thus suitable for small-scale testing of applications such as cDNA overexpression, gene knockdown, gene inhibition, or other application where stable, heritable expression is desired.

List of Components

Item	Catalog # (use to order full-sized products for larger-scale viral preps)	Amount
pPACKH1 HIV Lentiviral Packaging Plasmids	LV500A-1	200 µL
PEG-it Virus Precipitating Solution	LV810A-1	100 mL
PureFection Transfection Reagent	LV750A-1	1 mL
TransDux Virus Transduction Reagent	LV860A-1*	200 µL
TransDux MAX Virus Transduction Enhancer	LV860A-1*	10 mL
HEK293TN Producer Cell Line	LV900A-1	1 vial
Global UltraRapid Titering Kit	LV961A-1	1 Kit

*Components of the TransDux MAX Transduction Enhancer Kit Cat. No. LV860A-1

Storage

The kits are shipped on dry ice and should be stored at different temperatures as specified in the PAC provided with the kit. Properly stored kits are stable for 1 year from the date received.

General Information

To begin working with this kit, you should have your lentiviral vector selected and all cloning completed. This vector should be compatible with 3rd generation lentiviral packaging plasmids. Our pPACKH1 packaging mixture that is provided in the LentiSuite Deluxe Kit is a 3rd generation lentiviral system. The kit contains everything needed to go from lentiviral vector to packaged pseudovirus, generating titered, ready to infect pseudovirus for your experiments.

Protocol:

Required Materials

1. Your lentiviral construct (3rd generation lentivirus vector preferred)
2. Tabletop low speed centrifuge (e.g. Beckman GS-6R).
3. 100mm or 150mm plate for cell culture.

#Note: The expression construct being packaged should be purified using endotoxin-free plasmid purification kits to achieve maximal viral titer. The following kits are recommended for purification of your expression constructs:

- Qiagen EndoFree Plasmid Maxi Kit, Cat # 12362
- Macherey-Nagel NucleoBond Xtra Maxi EF Kit, Cat # 740424

Procedure

Pseudovirus production (100mm or 150mm plates)

The procedure below is for pseudoviral production for 100mm plates. For production in 150mm plates, please see the red text within the instructions below.

Day 1

1. Plate 3×10^6 (7-8 $\times 10^6$) 293TN cells in a fresh 100mm (150mm) plate in 10 mL (20 mL) of antibiotic-free DMEM medium (DMEM+FBS+Glu). For instructions on thawing and recovery of HEK293TN cells, please see the product analysis certificate (PAC) associated with the cells included in the LentiSuite Deluxe Kit.

Day 2

1. The cells should be 50 to 70% confluent at day of transfection
2. Add 0.8 mL (1.6 mL) of serum-free DMEM media into an Eppendorf tube. Add 2 μg (5 μg) of transfer plasmid and 20 μL (45 μL) pPACKH1-plasmid mix to the tube and mix by pipetting.
3. Add 24 μL (55 μL) of PureFection reagent to the tube, and vortex for 10 sec.
4. Incubate mixture at room temperature for a minimum of 15 min.
5. Add mixture drop-wise to the plate, and swirl to disperse evenly throughout.
6. Incubate plates in 37°C tissue culture incubator overnight.

Day 4 and 5

1. Collect the medium (which now contains pseudoviral particles) into 50-mL sterile, capped conical centrifuge tubes.
2. Centrifuge at 3000 x g for 15 minutes at room temperature to pellet cell debris.
3. Transfer the viral supernatant into new fresh tubes.

4. Add PEG-it at a final volume of 1:5. Example: 2 mL (5 mL) of PEG-it should be added to 8 mL (20 mL) of viral supernatant, invert 10 times to mix well. Keep everything cold from this point onwards. Store virus supernatant containing PEG-it at 4°C overnight, or up to 3 days.

Day 6

1. Harvest PEG-it precipitated virus by centrifuging at 4°C at 1500 x g for 30 min. Aspirate off the supernatant and resuspend the milky-white pellet in a small volume (1/100 to 1/1000 of original volume) using cold sterile PBS or cold DMEM.
2. Freeze virus aliquots at -80°C.

Transduction of Target Cells (24-well transduction)

Day 1

1. Plate 50,000 cells per well in a 24 well plate in culture medium.

Day 2

1. Cells should be between 50 to 70% confluent.
2. Aspirate medium from cells.
3. Combine TransDux™ and TransDux MAX Enhancer with culture medium to a final concentration of 1x. [Example: Add 2.5 µL of TransDux™ and 100 µL of MAX Enhancer to 400 µL culture medium and then transfer to each well].
4. Add virus to each well at different MOIs or different volumes, depending on experimental aims.
5. Incubate at 37°C for 72hrs.

Day 5

6. Look at the cells for reporter expression if the viral construct has a reporter like GFP and/or begin appropriate antibiotic selection to establish stable cell line. At this time, cells can be used for virus titering using our Global UltraRapid Titering Kit (see below).

Virus Titering

1. Aspirate off medium. Wash each well with PBS (at this point the plate can be frozen at -80°C).
2. Add 100µl of Lysis Buffer (SBI's UltraRapid Global Titering Kit) to each well.
3. Titer virus according to protocol given in the UltraRapid Global Titering Kit (SBI Cat# LV961A-1) – please see user manual in https://www.systembio.com/wp-content/uploads/manual_titer_kit_web-1.pdf

Next Steps and Related Products

Application	Related Products	Website links
Other Lentiviral Production Products		
Larger-scale lentiviral production	LentiSuite Basic and Deluxe Kit:	https://www.systembio.com/lentiviral-technology/delivery-systems/lentisuite/overview
Lentiviral production	HEK293TN Producer Cell Line	https://www.systembio.com/lentiviral-technology/delivery-systems/293tn-producer-cell-line/overview
Viral Titering	Global Ultra-Rapid Titering Kit	https://www.systembio.com/lentiviral-technology/delivery-systems/ultrarapid/overview
Lentiviral Production Controls	Positive Control Transduction Viruses	https://www.systembio.com/lentiviral-technology/delivery-systems/positive-transduction-controls/overview
Non-integrating Virus Packaging	Non-integrating Lentiviral System (pPACK-ID)	https://www.systembio.com/lentiviral-technology/delivery-systems/non-integrating
SBI Lentivectors		
Gene Delivery & Expression	SBI's 3 rd Generation Lentivector Collection	https://www.systembio.com/lentiviral-technology/expression-vectors

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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Licensing and Warranty Statement

Limited Use License

Use of the LentiSuite Kit (*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.

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- 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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