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# LentiStarter 3.0 Kit

Cat# LV060A-1

## User Manual

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Store kit at 4°C on receipt, do not freeze.

Version 2  
11/21/2017

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## Product Description

SBI's LentiStarter 3.0 Kit (Cat #LV060A-1) is a simple, cost-effective solution to generate your own lentivirus from 3<sup>rd</sup> generation lentiviral vectors. The kit contains all of the reagents necessary to produce pseudoviral particles (with exception of HEK293T/FT/TN producer cells, sold separately) from 5 x 100mm plates or 2 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	100 µL
PEG-it Virus Precipitating Solution	LV810A-1	20mL
PureFection Transfection Reagent	LV750A-1	120 µL
TransDux Virus Transduction Reagent	LV860A-1*	25 µL
TransDux MAX Virus Transduction Enhancer	LV860A-1*	1 mL

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

## Storage

The kits are shipped on blue ice and should be stored at +4°C upon receipt, do not freeze. Properly stored kits are stable for 1 year from the date received.

## General Information

SBI's LentiStarter Kit 3.0 is a great place to get started with lentiviral preparation, or this kit can be used to provide the reagents required for a small-scale lentiviral preparation. This kit is not intended for large-scale preparations – for these preps, we would recommend SBI's LentiSuite Basic (Cat #LV340A-1) or LentiSuite Deluxe (Cat #LV350A-1) Kits.

To begin working with this kit, you should have your lentiviral vector selected and all cloning completed. This vector should be compatible with 2<sup>nd</sup> and 3<sup>rd</sup> generation lentiviral packaging plasmids. Our pPACKH1 packaging mixture that is provided in the LentiStarter 3.0 is a 3<sup>rd</sup> generation lentiviral system. The kit contains everything needed to go from lentiviral vector to packaged pseudovirus, with the exception of the HEK293TN cells (sold separately).

## Protocol:

### Required Materials

1. Your lentiviral construct (3rd generation, preferred).
2. HEK 293T/FT/TN cells and suitable culture media for growing these cells.
3. Tabletop low speed centrifuge (e.g. Beckman GS-6R).
4. 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  $3 \times 10^6$  (7-8  $\times 10^6$ ) 293T/FT cells in a fresh 100mm (150mm) plate in 10 mL (20 mL) of antibiotic-free DMEM medium (DMEM+FBS+Glu).

#### 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  $\mu\text{g}$  (4  $\mu\text{g}$ ) of transfer plasmid and 20  $\mu\text{L}$  (45  $\mu\text{L}$ ) pPACKH1-plasmid mix to the tube and mix by pipetting.
3. Add 24  $\mu\text{L}$  (55  $\mu\text{L}$ ) 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 3

1. Replace transfection media with fresh complete growth media w/antibiotics

#### 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 10 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.

**OPTIONAL – 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).

## Next Steps and Related Products

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

## 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 LentiStarter 3.0™ 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.

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.

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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