pPACK-SPIKE™ Delta (B.1.617.2), SARS-CoV-2 "S" Pseudotype - Delta (B.1.617.2) Variant-Lentivector Packaging Mix

Cat. # CVD19-650A-1, CVD19-655A-1, CVD19-659A-KIT

User Manual

Storage: Store pPACK-SPIKE Packaging Mixes at -20˚ and PEG-it and PureFection at +4°C upon receipt

Version 1
7/15/2021

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Product Description

As potentially more transmissible variants of SARS-CoV-2 emerge, understanding how mutations in the spike protein impact SARS-CoV-2 behavior is critical. Designed to efficiently package most third-generation lentivectors, pPACK-SPIKE Delta (B.1.617.2), SARS-CoV-2 "S" Pseudotype - Delta (B.1.617.2) Variant – Lentivector Packaging Mix speeds and simplifies the preparation of lentiviral particles pseudotyped with the SARS-CoV-2 T19R, G142D, Δ156-157, R158G, L452R, T478K, D614G, P681R, D950N spike glycoprotein in place of VSV-G envelope protein (Cat.# CVD19-650A-1, CVD19-655A-1, CVD19-659A-KIT).

Based on SBI's highly cited pPACKH1 packaging system but with a truncated SARS-CoV-2 Spike protein[1] replacing the standard VSV-G envelope protein, pPACK-SPIKE Packaging Mixes consist of three plasmids that produce all of the structural and replication proteins needed to transcribe and package an RNA copy of expression lentivector into recombinant, "Spike" pseudotyped lentiviral particles. As a result, you can conduct a range of SARS-CoV-2 studies under BSL-2 conditions, including neutralization assays, studies of virus interactions with host surface proteins, and the development of vaccines and therapeutics.

For added convenience we also offer pPACK-BALD™, an envelope protein-free lentivector packaging mix that can be used as a negative control for pPACK-SPIKE studies, or for creating lentivirus particles pseudotyped with the envelope protein of your choice.

- Based on SBI's popular and highly cited pPACKH1 Packaging System
- Uses codon-optimized SARS-CoV-2 "S" protein from variant Delta (B.1.617.2, originally found in India) in place of VSV-G envelope protein
- Spike protein mutations are T19R, G142D, ΔE156-157, R158G, L452R, T478K, D614G, P681R, D950N
- Ideal for vaccine and antiviral efficacy studies under BSL2 conditions
- Package any 3rd-generation lentivector reporter of your choice, including SBI's popular LentiLabeler reporters
- Compare with our full range of pPACK-SPIKE S protein pseudotyped lentiviruses to better understand emerging variants
- Use with pPACK-BALD™, an envelope protein-free lentivector packaging system that is an ideal negative control

pPACK-SPIKE Delta (B.1.617.2), SARS-CoV-2 "S" Pseudotype - Delta (B.1.617.2) Variant - Lentivector Packaging Mix is available as stand-alone packaging mix with sufficient plasmids for 10 reactions (standard size) or 25 reactions (XL), as well as in convenient kit formats that include PureFection™ Transfection Reagent and PEG-it Virus Precipitation Solution.

TABLE 1. Available pPACK-SPIKE Delta (B.1.617.2), SARS-CoV-2 "S" Pseudotype - Delta (B.1.617.2) Variant- Lentivector Packaging Products

Catalog number	Description	Size
CVD19-650A-1	pPACK-SPIKE Delta (B.1.617.2), SARS-CoV-2 "S" Pseudotype - Delta (B.1.617.2) Variant – Lentivector Packaging Mix	10 Reactions
CVD19-655A-1	pPACK-SPIKE Delta (B.1.617.2), SARS-CoV-2 "S" Pseudotype - Delta (B.1.617.2) Variant – Lentivector Packaging Mix	25 Reactions
CVD19-659A-KIT	pPACK-SPIKE B.1.617.2 Combo Kit, includes Cat# CVD19-650A-1, plus PureFection Transfection Reagent (Cat# LV750A-1) and PEG-it VirusConcentration solution (Cat# LV810A-1)	1 Kit

Despite the safety features of third generation lentivectors and packaging system, use of HIV-based vectors falls within NIH Biosafety Level 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus, or the possibility of insertional mutagenesis. For a description of laboratory biosafety level criteria, consult the Centers for Disease Control Office of Health and Safety Web site at

http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm

It is also important to check with the health and safety guidelines at your institution regarding the use of lentiviruses and to always follow standard microbiological practices, which include:

- Wear gloves and a lab coat when handling the lentiviral vectors, pseudoviral particles, or transduced cells.
- Always work with pseudoviral particles in a Class II laminar flow hood.
- Perform all procedures carefully to minimize splashes, spills or the production of aerosols.
- Decontaminate work surfaces at least once a day or after any spill of viable material.
- *Decontaminate all cultures, stocks, and other regulated wastes before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory area should be placed in a durable, leakproof, properly marked (biohazard, infectious waste) container and sealed for transportation from the laboratory.

pPACK-SPIKE uses a simple, three-step workflow: Simply co-transfect the pPACK-SPIKE Plasmids and your lentivector construct into 293TN producer cells or other lentiviral production cells, isolate pseudoviral particles with an optional concentration step, and conduct your studies.

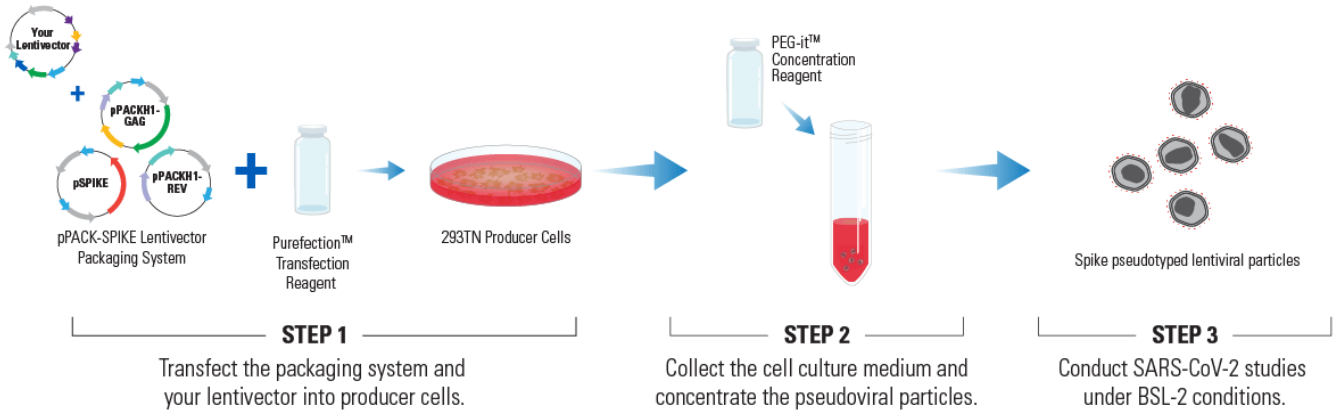

Figure 1. pPACK-SPIKE Workflow

REFERENCE

1. Ou X, *et al*. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat. commun*. 2020 Mar 27;11(1):1620. doi: 10.1038/s41467-020-15562-9. PMCID: PMC7100515.

List of Components

Table 2. Components for the individual pPACK-SPIKE Products

Catalog number	Components	Qty/Volume	Storage Temperature
CVD19-650A-1, LV550A-1	Packaging Plasmid Mix	200 µL	-20˚C
CVD19-655A-1, LV555A-1	Packaging Plasmid Mix	500 µL	-20˚C
CVD19-659A-KIT	pPACK-SPIKE Delta (B.1.617.2), SARS-CoV-2 "S" Pseudotype - Delta (B.1.617.2) Variant – Lentivector Packaging Mix	200 µl	-20˚C
	PureFection Transfection Reagent	1 ml	4˚C
	PEG-it Virus Concentration solution	100 ml	4˚C

Note: A reaction of packaging mix corresponds to co-transfections with a lentivector expression construct in 10-cm tissue culture plates (or, alternatively, 75 cm^2 flasks).

Additional Required and Optional Equipment Not Included in Kit

- **293TN Producer Cell Line** (Cat. # LV900A-1)
  To facilitate packaging, SBI offers a 293TN producer cell line that was optimized for effective production of a high titer of pseudoviral particles by introduction of the SV40 large T antigen and neomycin resistance gene. The 293TN cell line is a highly transfectable derivative of the HEK293 cell line with constitutive expression of SV40 T-antigen and neomycin resistance gene. It is comparable to the ATCC 293T/17 cell line. 293TN cells may be used in conjunction with the SBI Lentivector Packaging Kits to produce pseudotyped viral particles for transduction of target cell lines.

- **Dulbecco's Modified Eagle's Medium (D-MEM)**

  (high glucose with sodium pyruvate and L-glutamine; Invitrogen, Cat. # 11995073)

- **Fetal Bovine Serum** (Invitrogen, Cat. # 16000036)

- **Penicillin/Streptomycin** (Invitrogen, Cat. # 15070063)

- **Trypsin-EDTA** (Sigma, Cat. # T3924)

- **Tissue Culture Plates and Related Tissue Culture Supplies**

- **Sterile TE Buffer** (10 mM Tris pH 8.0, 0.1 mM EDTA pH 8.0)

For Catalog #s CVD19-650/655A-1 and LV550/555A-1 you will need to buy separately:

- **PureFection Transfection Reagent** (SBI. Cat # LV750A-1) PureFection is a powerful, broadly applicable transfection reagent for effective and reproducible transfections. PureFection reagent self-assembles nanoparticles in the presence of DNA and RNA. These complexes are readily taken up by target cells for efficient gene delivery. PureFection should be stored at 4°C upon receipt.

- **Peg-It Virus concentration solution** (SBI. Cat. # LV810A-1) PEG-*it*™Virus Precipitation Solution is a formulation of polyethylene glycol optimized for the precipitation of all lentiviral-based particles. It is 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.

Before you start:

We recommend using Stbl2 or OmniMax 2 T1R competent cells for transformation and propagation of the lentivector construct you wish to package into pseudoviral particles to avoid unwanted lentivector recombination events. (Recommended: MaxEfficiency Stbl2 competent cells, Life Tech (Cat # 10268-019) or One Shot OmniMAX 2 T1R competent cells, Cat. # C854003). Please do not use DH5 ala or Top 10 cells.

Expression constructs should be purified with a **QIAGEN Endotoxin-free Plasmid Purification Kit**. The following kit combinations can be used for Midi scale preparation of endotoxin-free DNA:

- QIAfilter Plasmid Midi Kit, Cat. # 12243, and EndoFree Plasmid Maxi Kit, Cat. # 12362
- QIAfilter Plasmid Midi Kit, Cat. # 12243, and EndoFree Plasmid Buffer Set, Cat. # 19048

Please visit the QIAGEN website to download the specialized protocol that is not contained in the user manual: **http://www1.qiagen.com/literature/protocols/pdf/QP15.pdf**

You will need 2.2-3.5 ug μg of lentiviral expression construct in sterile TE buffer with a concentration ranging from 0.2 – 2 μg/μl for each transfection in a 10-cm culture plate.

We recommend using low passage 293T cells for virus production.

Protocol

Transfection of 293TN Cells with PureFection™ reagent

To make lentivirus, cotransfect your lentivector construct with the pPACK-SPIKE or pPACK-BALD plasmids into 293TN cells using PureFection reagent. For some viruses, you may need to seed several plates of cells to obtain a high enough titer for transduction of target cells.

1. 18 - 24 hours prior to transfection, seed **7.0 – 8.0 x10^6** 293TN cells per 150 cm^2 cell culture plate* in 20 ml of normal culture medium (without antibiotics) so that the cell density reaches ~60 - 80% confluency at the time of transfection.
2. Add **1-1.6 ml** DMEM (serum free) to an autoclaved 2 ml Eppendorf tube.

   Add **45 µl** pPACK-SPIKE/pPACK-BALD and **5-8 µg** of your lentivector construct (<7 kb use 5 ug, 7 kb-9 kb use 6 ug, 9 kb-10 kb use 7 ug and >10 kb use 8 ug) into the DMEM. Mix by pipetting.
3. Add **55µl** PureFection into the same tube. Vortex for 10 seconds.
4. Incubate DMEM-Plasmid-PureFection mixture at room temperature for 15 minutes.
5. Add DMEM-Plasmid-PureFection mixture drop-wise to the dish, and swirl to disperse evenly throughout the plate.
6. Return the dish to the cell culture incubator at 37°C with 5% CO_2.
7. Change the medium 12-24 hours after transfection (optional).
8. At 48 hours and 72 hours after transfection, collect the medium (which now contains pseudoviral particles) into a 50-ml sterile, capped conical centrifuge tube. Centrifuge at 3000 x *g* for 15 minutes at room temperature to pellet cell debris. Transfer the viral supernatant into a new tube.

Caution: You are working with infectious pseudoviral particles at this stage. Please follow the recommended guidelines for working with BSL-2 safety class.

Concentrate viral particles with PEG-it™ Virus Precipitation Solution (*Optional)*

PEG-*it*™ 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 (4ºC) to every 4 volumes of Lentivector-containing supernatant. *(Example: 5ml PEG-it with 20ml viral supernatant).* 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.

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* If you use 10cm plates, seed 3-4X10^6 cells/ dish in 9 ml of normal culture medium without antibiotics.

- In step 2, 0.8ml of serum free medium should be used for each 10 cm plate.
- In step 3, 20µl of pPACK-SPIKE/pPACK-BALD and 2.2 -3.5 µg of plasmid should be used for each 10 cm plate.
- In step 4, 24 µl of PureFection should be used for each 10cm plate.

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 4ºC. 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 tub. 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 4ºC.
6. Aliquot in cryogenic vials and store at -70°C until ready for use.

Determine Pseudoviral titer by real time PCR using SBI's Ultra Rapid Lentiviral Titer Kit *(Optional)*

The Global UltraRapid Lentiviral Titer Kit is designed to rapidly determine the titers of infectious pseudoviral particles that are generated with SBI's FIV and HIV lentiviral vectors or libraries. It allows users to measure the copy numbers of integrated lentiviral constructs in genomic DNA of transduced target cells. The kit contains all components necessary for measuring the amounts of endogenous UCR1 DNA element and the pseudo-lentiviral-specific WPRE element that is integrated into the genomes of successfully transduced cells in each sample. The titer of each sample is then determined by calculating the amount of WPRE element relative to that of Ultra Conserved Region 1 (UCR1) DNA against a standard curve generated with the provided calibration standards. For titering, we recommend the use of any ACE2-expressing cells, including SBI's HEK293-ACE2 overexpression cells (Catalog # CVD19-200A-1).

We recommend that you titer the pseudovirus-containing supernatant before proceeding with transduction experiments for the following reasons:

- To ensure that pseudoviral stock is viable
- To determine the percentage of target cells which can be transduced with pseudoviral stock
- To control the number of copies of integrated viral constructs per target cell

Below are some key terms used in the protocol:

ifu/ml infectious units/ml	The relative concentration of infection-competent pseudoviral particles
MOI multiplicity of infection	The ratio of infectious pseudoviral particles (ifu) to the number of cells being infected. For example, if 1×10^6 cells are to be infected at an MOI of 0.1, then 1×10^5 ifu should be added to the cells
Transduction Efficiency	The average copy number of expression constructs per genome of target cell in the infected (transduced) population

For more information about SBI's Global UltraRapid Lentiviral Titer Kit, LV961A-1, please check the link below.

https://systembio.com/shop/ultrarapid-lentiviral-global-titering-kit-human-and-mouse-compatible/

Example Data and Applications

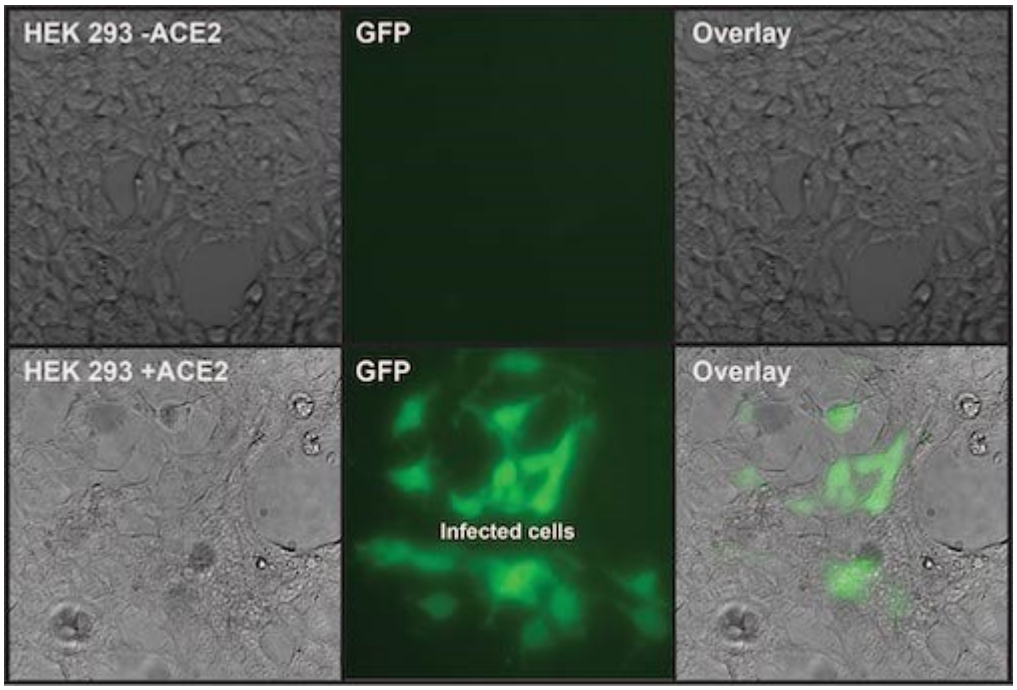

Figure 2. pPACK-SPIKE pseudovirus particles efficiently infect ACE2 overexpression cells. pPACK-SPIKE packaging mix was used to generate Spike pseudoviral particles carrying a GFP-reporter vector, concentrated using PEG-it Concentration Reagent, and transduced ACE2-overexpressing cells. GFP imaging shows expression of the GFP reporter only in cells overexpressing ACE2, demonstrating the SARS-CoV-2-like behavior of the Spike pseudoviral particles. Data courtesy of Henry David Herce and Michelle Prew from Dana-Farber Cancer Institute and Harvard Medical School.

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)
2438 Embarcadero Way
Palo Alto, CA 94303

Phone: (650) 968-2200
Toll-Free: (888) 266-5066
Fax (650) 968-2277

E-mail:
General Information: **info@systembio.com**
Technical Support: **tech@systembio.com**
Ordering Information: **orders@systembio.com**

Licensing and Warranty Statement

Limited Use License

Use of the pPACK-SPIKE and pPACK-BALD products (*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.

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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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System Biosciences (SBI)
2438 Embarcadero Way
Palo Alto, CA 94303

Phone: (650) 968-2200
Toll-Free: (888) 266-5066
Fax: (650) 968-2277

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