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ABP Lentiviral Packaging System

Catalog Number: D020-01, D020-02

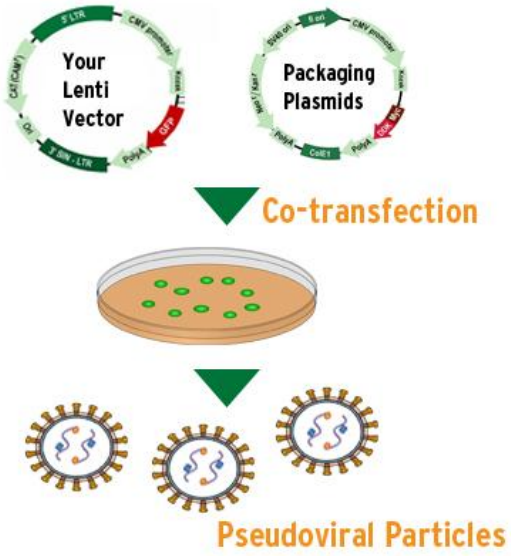
Table 1. Kit Components and Storage

Material	Amount	Storage	Stability
ABP Lentiviral Packaging System (Cat. No. D020-01, 10 assays)			The product is stable for at least one year when stored as directed.
Package Plasmid Mix	50 µL (0.5 µg/µL)	-20°C	
Control Plasmid	50 µL (0.25 µg/µL)	-20°C	
LipoFectMax™ Transfection Reagent	250 µL	4°C	
ABP Lentiviral Packaging System (Cat. No. D020-02, 40 assays)			
Package Plasmid Mix	200 µL (0.5 µg/µL)	-20°C	
Control Plasmid	100 µL (0.25 µg/µL)	-20°C	
LipoFectMax™ Transfection Reagent	1 mL	4°C	

Product Description

ABP Lentiviral Packaging System is designed to optimize the packaging of third generation lentivectors into virus particles which can express your lentiviral construct in a multitude of mammalian cells. The 3rd generation packaging system offers maximal biosafety as the lentiviral Rev gene is supplied as an independent vector from other structure genes, further eliminating the possibility of reverse recombination of vectors into replication competent viral particles.

ABP Lentiviral Packaging System includes packaging plasmid mix, eGFP positive control plasmid, and LipoFectMax™ Transfection Reagent.



Additional Materials Required

- HEK293T cell line (ATCC Cat #: CRL-11268)
- H1299 cell line (ATCC Cat #: CRL-5803)
- DMEM media (Mediatech Cat #: 10-013-CV)
- Fetal bovine serum (Biosera Cat #: FB-1001/500)
- Opti-MEM I Reduced-Serum Medium (Thermo Fisher Cat #: 31985062)
- Polybrene (ABP Biosciences Cat #: D025), Lentivirus Transduction Enhancer I (Cat #: D023) or Lentivirus Transduction Enhancer II (Cat #: D024)
- Penicillin-Streptomycin (Thermo Fisher Cat #: 15070063)
- BD Falcon® 5ml or 14 ml tubes (BD Falcon® Cat #:352053/352059)

Lentivirus Production Protocol

The following protocol provides optimized steps for lentivirus production in 293Ta packaging cells. The yield of recombinant lentiviral particles typically produced under these optimized conditions is 10 ml of 1–10 x10^6 infection units (ifu) per ml of un-concentrated supernatant from one 10-cm culture dish for eGFP positive controls. This amount of pseudoviral particles is generally sufficient to infect 1–10 x10^7 target cells at a MOI (multiplicity of infection) equal to 1. The titers of lentivirus decrease as the size of insert increases. Actual lentivirus titers for your gene of interest will vary accordingly.

Caution: Following this protocol results in the production of pseudoviral particles capable of infecting mammalian cells. The recommended guidelines for working with BSL-2 safety class must be adhered to.

1. Plate packaging cells

Two days before transfection, plate 1.3-1.5 x10^6 of HEK293T cells in a 10-cm dish in 10 ml of DMEM supplemented with 10% heat-inactivated fetal bovine serum so that the cells are 70–80% confluent at the moment of transfection. Incubate the cells at 37°C with 5% CO_2.

Note: Plating the HEK293T cells 2 days prior to transfection significantly increases the titer of lentivirus. Use heat-inactivated fetal bovine serum for lentivirus production.

2. Prepare DNA/Transfection Reagent complex

In a sterile polypropylene tube, dilute 2.5 µg of lentiviral ORF/shRNA/miRNA expression plasmid or control plasmid, and 5.0 µl (0.5 µg/µl) of Package Plasmid Mix into 200 µl of Opti-MEM® I Medium. In a separate tube, dilute 15 µl of LipoFectMax™ Transfection Reagent into 200 µl of Opti-MEM® I Medium. Add diluted LipoFectMax™ Transfection reagent dropwise to the DNA solution while gently vortexing the DNA-containing tube. Incubate the mixture for 20 minutes at room temperature.

Note: The DNA-LipoFectMax™ complex must be formed in the absence of proteins even though the complex is able to transfect cells in the presence of proteins such as 10% serum. Opti-MEM® I Medium is recommended for diluting both DNA and LipoFectMax™ Transfection reagent. Serum-free DMEM can be used in place of Opti-MEM® I Medium but the transfection efficiency will be compromised.

3. Transfect packaging cells

Add the DNA-LipoFectMax™ complex directly to each dish and gently swirl the dish to distribute the complex. Incubate the cells in a CO_2 incubator at 37°C overnight (8–14 hours). Replace the overnight culture medium with fresh DMEM medium supplemented with 2–5% heat-inactivated fetal bovine serum and penicillin-streptomycin, and continue incubation in the CO_2 incubator at 37°C.

4. Harvest lentivirus

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After 48 hours post transfection, collect the pseudovirus-containing culture medium in sterile capped tubes and centrifuge the tubes at 500 x *g* for 10 minutes to get rid of cell debris. Following centrifugation, filter the supernatant through 0.45 µm polyethersulfone (PES) low protein-binding filters.

Note: If you want to collect more lentivirus, replace lentiviral containing supernatants with fresh DMEM medium supplemented with 2–5% heat-inactivated fetal bovine serum, penicillin-streptomycin. Lentivirus containing medium can be collected again at 72 hours post-transfection. Do not use nitrocellulose filters as nitrocellulose is known to bind lentivirus and reduce titers.

The supernatant containing lentiviral particles can be used directly to determine the titer and to transduce target cells in vitro as long as the target cells can survive in conditioned medium. Lentiviral stocks should be aliquoted and stored at -80°C. Expect significant loss of viral titer with each freeze/thaw cycle.

Lentivirus Titration

The titer of harvested lentiviral stocks can be determined by commercial qPCR Lentiviral Titration Kits.

Transduction of Target Cells with Lentiviruses

The transduction efficiency of mammalian cells varies significantly under different experimental conditions. This includes virus concentration, exposure time to the virus and growth area of the well or plate used for the infection.

To determine the viral concentration required to provide the desired multiplicity of infection (MOI) for your target cells, it is advisable to perform several test transductions with reporter viral particles at a range of different volumes such as 1 µl, 5 µl, 10 µl, and 100 µl. Results from these preliminary tests can be used to determine an optimal concentration that will yield the highest percentage of successfully infected cells.

The following protocol has been provided as a general guideline only, to be used as a starting point for determining optimal conditions for target cell transduction:

1. Plate the target cells in a 24-well plate, 24 hours prior to viral infection at a density of 5×10^4 cells per well. Add 0.5 ml of complete optimal medium (with serum and antibiotics if required) and incubate the cells at 37°C with 5% CO_2 overnight.
2. For each well, prepare 0.5 ml of virus suspension diluted in complete medium with polybrene at a final concentration of 8 µg/ml.

   Note: Use several dilutions of pseudoviral stock (0.1 µl to 100 µl). We recommend gradient dilution of 0.1 µl, 0.3 µl, 3 µl,10 µl, 30 µl for standard particles, and 0.1 µl, 0.3 µl, 0.5 µl, 0.7 µl, 0.9 µl for purified particles. Mix the virus with the medium gently by inverting the tubes several times. Do not vortex.
3. Infect the target cells by removing the old culture medium and replacing it with 0.5 ml of diluted viral supernatant. Place the plates in a 37°C incubator with 5% CO_2 and incubate cells overnight. If the transduction efficiency of the target cells is low, try Lentivirus Transduction Enhancer I (Cat #: D023), or Lentivirus Transduction Enhancer II (Cat #: D024).
4. Replace the old medium with 0.5 ml of fresh complete medium (without Polybrene).
5. The infected target cells can be analyzed for transient expression of transgenes using an appropriate biological assay. If you have used an internal eGFP control, determine the percentage of infected cells with a fluorescent microscope.
6. Once an effective MOI has been determined for the target cells through preliminary test infections, use the appropriate volume of virus to infect your cells.