horizon

TECHNICAL MANUAL

Dharmacon™ Trans-Lentiviral packaging kits

Product description

The Dharmacon™ Trans-Lentiviral™ Packaging System efficiently generates replication-incompetent lentiviral particles to deliver and express an shRNA or ORF (open reading frame) construct in either dividing or non-dividing mammalian cells[1]. Among commercially available lentiviral vector systems, the Trans-Lentiviral Packaging System offers a superior safety profile, as the packaging components are separated onto five plasmids. A detailed description of the Trans-Lentiviral Packaging System can be found in Wu[2]. Kits for 10 packaging reactions are available (with and without Dharmacon HEK293T producer cells), as well as larger bulk sizes for 50 and 100 packaging reactions.

See Appendix A for detailed kit descriptions, component storage and shipping conditions.

Components of the Dharmacon Trans-Lentiviral packaging system

1. **Trans-Lentiviral packaging mix**
   The packaging mix contains an optimized mixture of five packaging plasmids to facilitate viral packaging of the transfer vector following co-transfection into HEK293T producer cells[3,4]. These plasmids supply the helper functions as well as structural and enzymatic proteins in trans required to produce lentiviral particles. For more information about the components for the packaging plasmids, see the section entitled Biosafety Features of the Trans-Lentiviral Packaging System.
2. **Calcium Phosphate Transfection Reagent**
   Calcium chloride, when complexed with DNA and then co-precipitated by adding phosphate buffer, can facilitate uptake of DNA in transformed, adherent cells. The kit includes calcium chloride and 2x HEPES-buffered saline solution (2x HBSS) which have been stringently tested for optimal pH and stability. Highly efficient calcium phosphate transfection of the transfer and packaging vectors is obtained specifically in HEK293T cells
3. **Dharmacon HEK293T Packaging Cell Line (provided as an option with 10 reaction kit or available for separate purchase)**
   HEK293T cells are ideal for packaging lentiviral particles and can yield high titers following co-transfection of the Trans-Lentiviral packaging mix with a Dharmacon™ shRNA or ORF transfer plasmid. These cells stably express the SV40 Large-T Antigen (Simian Vacuolating Virus 40 TAg) which allows high levels of protein to be expressed from vectors containing the SV40 origin of replication.
4. **Control Vectors (provided in 10-reaction Trans-Lentiviral packaging kits)**
   A Dharmacon™ GIPZ™ Non-silencing Control is provided in the shRNA packaging kits. The non-silencing control expresses the TurboGFP™ (Evrogen, Moscow, Russia) reporter gene and an shRNA sequence within the Cold Spring Harbor miR-30 expression scaffold with no homology to known mammalian genes.

Cat #	10 Reaction Kit	10 Reaction Kit (with Dharmcon HEK293T cells)	50 Reaction Kit	100 Reaction Kit
Dharmacon Trans-Lentiviral shRNA Packaging Kit with Calcium Phosphate Transfection Reagent	TLP5912	TLP5917	TLP5913	TLP5914
Dharmacon Trans-Lentiviral ORF Packaging Kit with Calcium Phosphate Transfection Reagent	TLP5916	TLP5918	TLP5919	TLP5920

5. **A Precision Lenti-ORF RFP Control is provided in the ORF packaging kits**
   The Precision LentiORF RFP Control expresses the TurboRFP (Evrogen, Moscow, Russia) reporter gene in place of a human ORF sequence

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Biosafety features of the Dharmacon Trans-Lentiviral packaging system

The Dharmacon Trans-Lentiviral Packaging System offers a superior safety profile compared to other commercially available lentiviral vector systems. The packaging components are separated onto five plasmids (Figure 1). Additionally, expression of gag-pro and tat-rev are under the control of the conditional tetracycline-responsive promoter element (TRE), limiting expression of these viral components strictly to cells expressing the tetracycline transactivator. A detailed description of the Trans-Lentiviral Packaging System can be found in Wu[2].

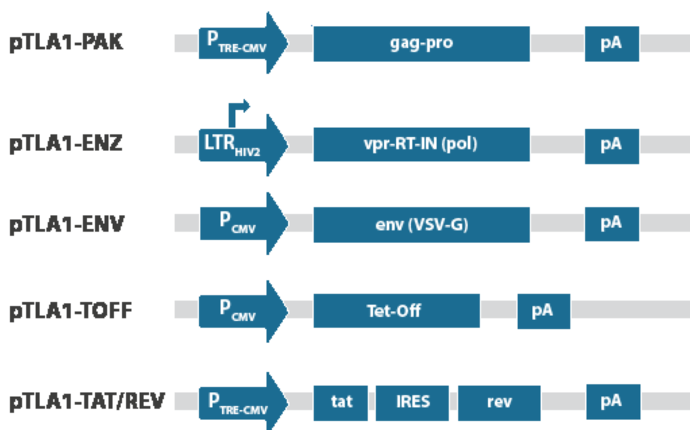

Figure 1. Plasmid vectors in the Dharmacon Trans-Lentiviral packaging system

The five plasmid components for the Dharmacom Trans-Lentiviral Packaging System are as follows:

1. **pTLA1-PAK**
   The gag-pro packaging construct contains a DNA fragment from the HIV-1 molecular clone SG3 (coordinates 258 to 8384), which has been modified by a 39-base deletion of the packaging signal (Ψ) and a 1357-base deletion in the env gene (coordinates 5827 to 7184). The pol open reading frames (ORFs) for the RT and IN genes were deleted by replacing the first codon of each with stop codons (TAA). A frame-shift mutation was introduced into the Vpr-encoding sequence by blunt-end ligation at the SalI restriction site. Together these modifications delete the expression of RT, IN, as well as the accessory proteins, Vpr, Vif, Vpu, and Nef. Expression of Gag-Pro is controlled by a TRE promoter.
2. **pTLA1-ENZ**
   The RT and IN proteins are packaged in the viral particle by an in-frame fusion with the virion-associated protein Vpr. Expression of the Vpr-RT-IN fusion polyprotein is under the control of the HIV-2 LTR, which is trans-activated by the Tat protein.
3. **pTLA1-ENV**
   The VSV-G expression vector contains the VSV-g cDNA expressed from a human cytomegalovirus (CMV) immediate early promoter. In addition, an SV40 polyadenylation signal has been cloned downstream of the VSVg cDNA.
4. **pTLA1-TOFF**
   The Clontech™ Tet-Off™ expression vector contains the tetracycline-controlled transactivator (tTA) under the control of the hCMV immediate early promoter. Tet-Off is required for transcriptional activation of Gag-Pro expression (pTLA1-PAK) and TAT/REV expression (pTLA1-TAT/REV).
5. **pTLA1-TAT/REV**
   The HIV-1 auxiliary proteins Tat and Rev are expressed from a bicistronic vector containing an Internal Ribosomal Entry Site (IRES). Its expression is under control of the TRE promoter. Tat expression is required for transactivation of the 5′ LTRs found in GIPZ shRNA, Precision LentiORF, and pTLA1-ENZ viral vectors. Rev is required for nuclear export and translation of mRNA transcripts encoding the gag-pro and vpr-RT-IN (pol) genes, as well as the full-length viral genome.

General containment considerations

When using the Dharmacon Trans-Lentiviral Packaging System to create lentiviral particles, the researcher is generating a product that may safely be utilized under the appropriate laboratory safety guidelines to silence or promote gene expression in a wide range of mammalian cells. The major risks associated with the use of lentiviral vectors are (1) the potential for generating replication- competent lentiviral particles, and (2) the potential for oncogenesis due to insertional activation/inactivation of key regulatory genes; for example, activation of an oncogene or inactivation of a tumor suppressor.

To mitigate these risks, either BSL-2 or enhanced BSL-2 containment is required when handling any lentiviral vector particles. For guidance on containment for lentiviral vectors, please refer to the Recombinant DNA Advisory Committee (RAC) guidelines for research with lentiviral vectors, downloadable from: https://osp.od.nih.gov/wp-content/uploads/Lenti_Containment_Guidance.pdf

In the U.S., download the U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health, Biosafety in Microbiological and Biomedical Laboratories (BMBL), downloadable here: https://www.cdc.gov/biosafety/publications/bmbl5/.

See also: NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines), downloadable here: https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.html.

References

1. Naldini, L. *et al.*, In Vivo Gene Delivery and Stable Transduction of Nondividing Cells by a Lentiviral Vector. *Science* **272**, 263–267 (1996).
2. Wu, X. *et al.*, Development of a Novel Trans-Lentiviral Vector That Affords Predictable Safety. *Molecular Therapy* **2**, 47–55 (2000).
3. Kappes J.C., Wu X, Safety considerations in vector development. *Somat Cell Mol Genet* **26**, 147-58 (2001).
4. Kappes J.C. *et al*, Production of trans-lentiviral vector with predictable safety. *Methods Mol Med* **76**, 449-65 (2003).

Protocols

I. Producing Lentiviral Particles in HEK293T Cells

Below are protocols for the use of Trans-Lentiviral Packaging Kits to generate lentiviral particles. The production of lentiviral particles begins with co-transfection of the Dharmacon Trans-Lentiviral packaging mix with an shRNA or ORF transfer vector into HEK293T packaging cells, using the calcium phosphate reagent. Following co-transfection, replication-incompetent virions are released into the medium for collection and downstream use. The examples given below are optimized for the production of average-titer (≈10^6 TU/mL) lentiviral particles in either 6-well cell or 100 mm cell culture plates, followed by concentration by ultracentrifugation to yield high-titer (≈108 TU/mL) lentiviral particles.

Some general guidelines for successful co-transfection of transfer vector and packaging plasmid DNA include the following:

- It is important to use exponentially growing cells that are regularly passaged and actively dividing. The highest transfection efficiencies are achieved if cells are plated the day before transfection. Adequate time should be given to allow the cells to recover from the passaging (generally > 12 hours). Prior to transfection, ensure that Dharmacon HEK293T cells are in a rapid replication state by growing cells to approximately 70% confluency in a cell culture dish and passaging at a 1:2 ratio for at least two consecutive days. Preparing cells in this manner will help to ensure consistent and maximum lentiviral particle yield.
- Plasmid DNA with low purity may transfect poorly due to inhibitory effects of contaminants. Avoid cytotoxic effects by carefully preparing and purifying your plasmid DNA. Determine the quality of your DNA using 260 nm/280 nm absorbance. Generally a ratio of 1.8-2.0 is considered acceptable.

Cell Plating

Prepare cells in at least one well of a 6-well plate or one 100 mm cell culture plate for each transfer vector to be packaged into lentiviral particles.

- Trypsinize and count HEK293T cells.
- Dilute cells in normal growth medium (DMEM High Glucose, Sodium Pyruvate, 10% FBS, 1% Pen/Strep) to achieve the optimal cell density for the chosen cell culture plate; optimal cell confluency at time of transfection should be 85-95%.
  - » a. For each well of a 6-well plate, prepare 1.2×10^6 cells in 2 mL.
    b. For each 100 mm cell culture plate, prepare 5.5×10^6 cells in 14 mL.
- Incubate cells at 37 °C with 5% CO_2 overnight.

Transfection

Thaw $CaCl_2$ and 2x HBSS briefly in a 37 °C water bath. After thawing, both reagents may be stored for several weeks at 4 °C without detectable loss of function. Reagents should be brought to ambient temperature prior to proceeding with transfection.

1. For each well of a 6-well plate or 100 mm cell culture plate, prepare the indicated quantity of transfer vector DNA and Trans-Lentiviral Packaging Mix in a 5 mL (Fisher Scientific Cat #14-959-1A) or 50 mL (Fisher Scientific Cat #14-432-22) polystyrene tube. Use sterile water to bring DNA mix to the indicated total volume.

	Lentiviral transfer vector DNA (shRNA or ORF)	Trans-Lentiviral packaging mix	Total volume (with sterile water)
One well of a 6-well plate	6 µg	4.3 µL	135 µL
100 mm plate	42 µg	30 µL	945 µL

2. Add the indicated volume of $CaCl_2$ to the diluted DNA above:

	$CaCl_2$
One well of a 6-well plate	15 µL
100 mm plate	105 µL

3. Vortex the tube at a speed sufficient to thoroughly mix reagents without spillover. While vortexing, add drop-wise the indicated volume of 2x HBSS:

	2x HBSS
One well of a 6-well plate	150 µL
100 mm plate	1050 µL

4. Incubate at room temperature for 3 minutes. A light chalky precipitate should appear during this incubation (the precipitate may not always be obvious).
5. Add the total volume (300 µL or 2.1 mL) of transfection mix drop-wise to the cells. **Note: The exact volume may be slightly less due to pipetting loss, but this will not negatively impact transfection efficiency.**
6. Incubate cells at 37 °C with 5% CO_2 for 10-16 hours (do not extend this time).
7. After > 16 hours of incubation, examine the cells microscopically for the presence of a fluorescent reporter protein, such as Evrogen TurboGFP, if one is expressed by the lentiviral transfer vector, as an indicator of transfection efficiency. **Note: The color of the medium may be orange or orange/yellow; this does not affect viral production.**
8. Prepare reduced serum medium:
   - » a. High Glucose DMEM (Fisher Scientific Cat #SH30243.LS)
     b. 5% Fetal Bovine Serum (Fisher Scientific Cat #SH30070.03)
     c. 2 mM L-glutamine (Fisher Scientific Cat #SH30034.01)
     d. 1x Penicillin/Streptomycin (Fisher Scientific Cat #SV30010)
9. Remove calcium phosphate-containing medium from cells and replace with the indicated volume of reduced serum medium:

	Reduced Serum Medium
One well of a 6-well plate	2 mL
100 mm plate	14 mL

10. Incubate cells at 37 °C with 5% CO_2 for an additional 48 hours.

Viral particle collection and concentration

1. Harvest viral particle-containing supernatants 48 hours after the medium change by removing medium to a 15 mL sterile, capped, conical tube. **Caution: Remember that you are working with infectious, though non-replicating, viral particles at this stage. Follow the recommended guidelines for working with BSL-2 biological organisms.**
2. Pellet non-adherent cells and debris by centrifugation at 1600 × g at 4 °C for 10 minutes to pellet cell debris.

3. For average-titer, unconcentrated viral particles generated in either a 6-well or 100 mm plate, slowly remove supernatant and transfer to a fresh tube. Mix by pipetting and aliquot lentiviral particles as needed. Prepare one small aliquot (~ 20 µL) for titer determination. Always store lentiviral particles at -80 °C.
4. To produce concentrated high-titer viral particles, we recommend a filtration step in which the supernatant is passed through a sterile, 0.22-0.45 µM low protein binding filter (such as, Millipore Millex-HV 0.45 µM PVDF filters) after the low-speed centrifugation step to remove any remaining cellular debris. Concentrate by ultracentrifugation in a swinging-bucket ultracentrifuge rotor. Transfer the filtered supernatant to a sterile ultracentrifuge tube. Bring volume to the required minimum volume of your rotor buckets with DMEM containing no serum. For an SW28 rotor, centrifuge at 23,000 rpm for 1.5-2 hours at 4 °C.
5. After centrifugation, carefully aspirate and discard the supernatant.
6. Pipette the desired resuspension volume of DMEM (no serum) onto the pellet at the bottom of the tube.
7. The visible pellet is made up mostly of serum proteins from the culture media of the transfected cells. The viral particles need to be dislodged from this protein pellet. After adding the DMEM to the pellet, incubate for 5-10 minutes at 4 °C. Then gently pipette up and down about 30 times, avoiding the formation of bubbles.
8. Transfer the resuspended pellet to a sterile microfuge tube and centrifuge at full speed for 3-4 minutes. This centrifugation will pellet the serum proteins, which adhere to the bottom of the tube. After centrifugation, transfer the supernatant to a new microfuge tube and aliquot as needed. Prepare one small aliquot (~ 20 µL) for titer determination. Always store lentiviral particles at -80 °C.

Determining titer of lentiviral particle stocks

It is important to determine titers of the lentiviral particle stocks not only in the producer cells (such as HEK293T) but also in the downstream experimental mammalian cell line of choice. This protocol may be used to determine the titer of any lentiviral vector that expresses a reporter gene, such as Evrogen TurboGFP or TurboRFP. (If the reporter is under the control of a Tet-inducible promoter system, as with Dharmacon™ TRIPZ™ shRNA products, be sure to include doxycycline in the cell culture medium.)

1. The day before transduction, seed a 24-well tissue culture plate with HEK293T cells at 5×10^4 cells per well in DMEM (10% FBS, 1% Pen/Strep). The following day, the wells should be no more than 40-50% confluent. The cell plating density will need to be determined empirically for other cell lines.
2. Make dilutions of the viral stock in a round bottom 96-well plate using serum-free medium. Utilize the plate as shown in Figure 2 using one row for each viral particles stock to be tested. Use the procedure below (starting at step 4) for dilution of the viral stocks. The goal is to produce a series of five-fold dilutions to reach a final dilution of 390,625-fold.
3. To each well add 80 µL of serum-free medium.
4. Add 20 µL of thawed viral particles stock to each corresponding well in column 1 (five-fold dilution). Pipette contents of well up and down 10-15 times. Discard pipette tip.
5. With new pipette, transfer 20 µL from each well of column 1 to the corresponding well in column 2. Pipette contents of well up and down tips 10-15 times and discard pipette tips.
6. With new pipette tips, transfer 20 µL from each well of column 2 to the corresponding well in column 3. Pipette contents of well up and down 10-15 times and discard pipette tip.
7. Repeat transfers of 20 µL from columns 3 through 8, pipetting up and down 10-15 times and changing pipette tips between each dilution. It is strongly recommended that you use a high quality multichannel pipettor when performing multiple dilutions. Pre-incubate the dilutions of the viral particles stock for 5 minutes at room temperature.
8. Label a 24-well plate as shown in Figure 3 using one row for each viral particles stock to be tested.
9. Remove culture medium from the cells in the 24-well plate.
10. Add 225 µL of serum-free medium to each well.
11. Transduce cells by adding 25 µL of diluted viral particles from the original 96-well plate (Figure 2) to a well on the 24-well destination plate (Figure 3) containing the cells. For example, transfer 25 µL from well A2 of the 96-well plate into well A1 in the 24-well plate (Table 1).
12. Incubate transduced cultures at 37 °C for 4 hours.
13. Remove transduction mix from cultures and gently add 1 mL of DMEM (10% FBS, 1% Pen/Strep). For Tet-inducible lentiviral transfer vectors, such as TRIPZ, include 1 µg/mL doxycycline in the medium.
14. Culture cells for 72 hours.
15. Choose a well in the destination plate with a reasonable number of fluorescent cells to count the TurboGFP or TurboRFP expressing colonies of cells. Count each multi-cell colony as one transduced cell, as the cells will be dividing over the 48 hour culture period. Figure 4 illustrates this principle.
16. Transducing units per milliliter (TU/mL) can be determined using the following formula: # of Evrogen TurboGFP or TurboRFP positive colonies counted × dilution factor × 40 = #TU/mL.
    - **Example: 55 Evrogen TurboGFP positive colonies counted in well A3.**
    - **55 (Evrogen TurboGFP positive colonies) × 625 (dilution factor) × 40 = 1.38×10^6 TU/mL**

    The titer units are given in transducing units (TU) per mL, so the number 40 is used to convert the 25 µL used in the titration ("volume of diluted virus used," Table 1) to one milliliter.

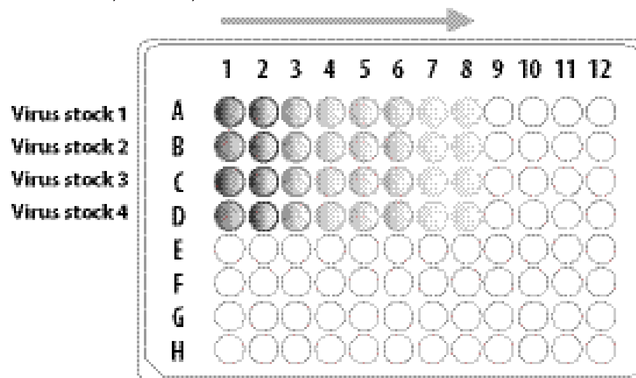

Figure 2. Five-fold serial dilutions of virus stock

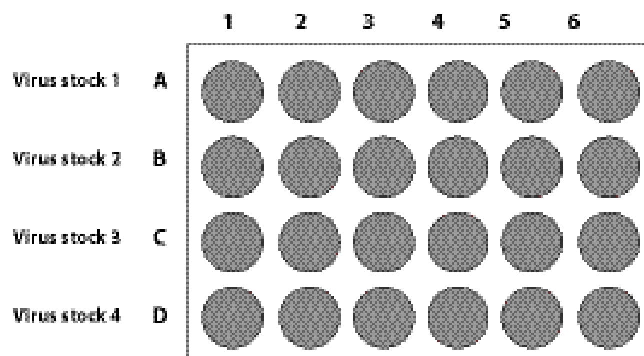

Figure 3. Twenty-four well tissue culture plate, seeded with Dharmacon HEK293T cells, used to titer the virus

Table 1. Creating a dilution series of viral particles.

Well			
Originating (96-well plate)	Destination (24-well plate)	Volume of diluted viral particles used	Dilution factor
A1		25 µL	5*
A2	A1	25 µL	25
A3	A2	25 µL	125
A4	A3	25 µL	625
A5	A4	25 µL	3125
A6	A5	25 µL	15625
A7	A6	25 µL	78125
A8		25 µL	390625*

*Please note that when expecting very low or very high titers, it would be advisable to include either well 1 or well 8, respectively

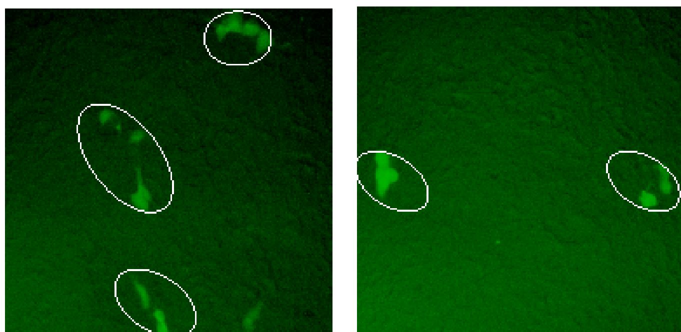

Figure 4. Determining viral titer by colony counting. HEK293T cells were transduced with a serial dilution of Dharmacon™ GIPZ™ Lentiviral particles. Cells were observed by fluorescence microscopy 72 hours post-transduction. Each circle represents an individual colony.

Frequently asked questions

When using the calcium phosphate reagent to co-transfect my transfer vector and the Dharmacon Trans-Lentiviral packaging mix, all of my HEK293T cells died after transfection – what happened? Some cell death is commonly observed 36-48 hours post-transfection. Lethality near the time of viral harvest is actually a positive sign that lentiviral particles are being actively produced. High expression of Trans-Lentiviral packaging mix components is likely the cause. Conversely, a high degree of cell death should not be observed in less than 24 hours after transfection. This is often due to handling errors during transfection. Cells can become dislodged during the addition of the transfection reagent and changing the medium; be sure to add reagents slowly and gently to the cells. Make sure the confluency prior to transfection is 85-95% and handle the plates with care while transporting from the cell culture hood to the incubator.

Why does the morphology of my HEK293T cells change following transfection? Expression of the VSV-g glycoprotein causes HEK293T cells to fuse, resulting in the appearance of multinucleated syncytia. This morphological change is normal and does not affect production of the lentiviral particles.

Does the order in which each reagent is added to generate the calcium phosphate-DNA complex make a difference? We have optimized the transfection protocol by adding the reagents in the order indicated. Transfections may still be successful if the order of reagent addition is changed, but we recommend assembling the transfection reaction in the order indicated in the protocol.

Is vortexing the sample while adding HBSS really necessary? No, vortexing the DNA reaction mix is not essential, but should help to achieve the highest possible transfection efficiency.

How long can the calcium phosphate-DNA complex incubate at room temperature? Precipitation of DNA by calcium phosphate is time and temperature dependent [M. Jordan, A. Schallhorn, Transfecting mammalian cells: optimization of critical parameters affecting calcium-phosphate precipitate formation. *Nucleic Acids Res.* **24**, 596-601 (1996)]. From our experience, optimal transfection efficiency occurs approximately 3 minutes after addition of HBSS.

What efficiency should I expect from transfection of DNA into HEK293T cells using calcium phosphate? We normally obtain 90-95% transfection efficiency.

What viral titer should I expected from harvested supernatants? The achievable viral titer will vary between transfer vectors. When packaging GIPZ shRNA constructs, we typically obtain titers of ≥ 10^6 TU/mL. Following the protocol described here for concentrating lentiviral particles we generally are able to obtain titers in the range of 10^8 TU/mL. We are often able to achieve titers of ≥ 10^9 TU/mL by increasing the number of transfected plates and/or reducing the resuspension volume.

Which Millipore filters do you use for the filtration of viral supernatant in preparation for concentration of particles? We use various filters dependent on volume to be filtered:

- ≤ 50 mL: Millipore Cat #SCGP00525, Steriflip™-GP Filter Unit, 0.22 µM PES membraneOr, Millipore Cat #SE1M003M00, Steriflip-HV Filter Unit, 0.45 µM PVDF membrane
  ≤ 150 mL: Millipore Cat #SCGPU01RE, Stericup-GP Filter Unit, 0.22 µM PES membrane
- ≤ 500 mL: Millipore Cat #SCGPU05RE, Stericup-GP Filter Unit, 0.22 µM PES membrane
- ≤ 1000 mL: Millipore Cat #SCGPU10RE, Stericup-GP Filter Unit, 0.22 µM PES membrane

I don't have the same kind of rotor mentioned in your protocol for concentrating virus, how do I know mine will work? The use of an SW28 rotor system is not required; other rotor sizes are also appropriate. If using an alternative rotor, cross reference the g force and time of run for your rotor.

Additionally, the following journal article references the use of a SW32Ti rotor (19500 rpm for 2 hour) to concentrate lentivirus:

F. Di Nunzio, B. Piovani, Transduction of human hematopoietic stem cells by lentiviral vectors pseudotyped with the RD114-TR chimeric envelope glycoprotein. *Hum. Gene Ther.*, **18**, 811-820 (2007).

There is a conversion for rpm to rcf at this website: http://insilico.ehu.es/mini_tools/rcf_rpm.php

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Oxford technology

PCT Application (filing date)	Priority Application(s) (filing date)	National Application	Issued Patents
	US 07/586,603 (21 Sept 1990)	US 08/361,839	5,817,491
PCT/US91/05699 (9 Aug 1991)	US 07/658,632 (19 Feb 1991)	EP 91915104.3 JP 3-514518 AU 84302/91 CA 2,104,396	0572401 3547129 663470 2,104,396
	US 07/1 70,515 (21 March 1988) US 07/1 70,515 (21 March 1988)	US Continuation 08/156,789 US Continuation 08/462,492 US Continuation 10/205,179	5,591,624 5,716,832
PCT/US91/06852 (20 Aug 1991) WO 92/05266	US 07/586,603 (21 Sept 1990)	AU 88424/91 AU Divisional 47984/96	665176 690427
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Benitec technology licensed patents

Name Inventor	Title	Patent No.	Application No.
Graham/Rice	Control of Gene Express	ZA 2000/4507* AU2001100608* SG75542* US 6,573,099* GB2353282* AU 743316* NZ 506648* US 10/346853* PCT/AU/99/00195* BR PI9908967-0* CA 2323726* CB 99804255-2* CZ PB2000-3346* EP 99910039.9* US10/646,070 US 10/759,841* EP 04015041.9* AU2005211538	HK 01105904.3* HU PO101225* IN 2000/00 169/DEL* JP P2000-537990 KR 7010419/2000* MX008631* PL P.343064* SK PV 1372-2000* AU 35647/02* NZ 525941* SG200205122-5* US 09/646807* PP2492/98* AUPP2499/98* US 10/821,710* US 10/821,726* AU 2005209648
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*Indicates a "Co-Owned Patents" are jointly owned by Benitec and CSIRO.

Appendix A

Dharmacon Trans-Lentiviral ORF Packaging Kit with Calcium Phosphate Transfection Reagent

Kit contents	TLP5916 (10 rxn)	TLP5918 (10 rxn + cells)	TLP5919 (50 rxn)	TLP5920 (100 rxn)	Shipping condition	Storage
Dharmacon Trans-Lentiviral Packaging Mix	285 µg	285 µg	285 µg × 5	285 µg × 10	Wet ice	-20 °C
Dharmacon HEK293T Packaging Cell Line	not included	1.0 mL	not included	not included	Dry ice	Liquid N_2
$CaCl_2$ Reagent	1.2 mL	1.2 mL	6 mL	6 mL × 2	Wet ice	-20 °C
2x HBSS Reagent	12 mL	12 mL	60 mL	60 mL × 2	Wet ice	-20 °C
Dharmacon Precision LentiORF RFP Control DNA	45 µg (0.45 µg/µL)	45 µg (0.45 µg/µL)	not included	not included	Wet ice	-20 °C

Dharmacon Trans-Lentiviral shRNA Packaging Kit with Calcium Phosphate Transfection Reagent

Kit contents	TLP5912 (10 rxn)	TLP5917 (10 rxn + cells)	TLP5913 (50 rxn)	TLP5914 (100 rxn)	Shipping condition	Storage
Dharmacon Trans-Lentiviral Packaging Mix	285 µg	285 µg	285 µg × 5	285 µg × 10	Wet ice	-20 °C
Dharmacon HEK293T Packaging Cell Line	not included	1.0 mL	not included	not included	Dry ice	Liquid N_2
$CaCl_2$ Reagent	1.2 mL	1.2 mL	6 mL	6 mL × 2	Wet ice	-20 °C
2x HBSS Reagent	12 mL	12 mL	60 mL	60 mL × 2	Wet ice	-20 °C
Dharmacon GIPZ Non-Silencing Control DNA	45 µg (0.45 µg/µL)	45 µg (0.45 µg/µL)	not included	not included	Wet ice	-20 °C

If you have any questions, contact

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