



## Pellet-Down™ Lentivirus Concentration Kit-User Manual

Catalog#	Kit Components	Amounts
<u><b>LV-CONC</b></u>	Pellet-Down™ Concentration solution	200 ml (4x solution, filter-sterilized)
	Virus Suspension Solution	10 ml (filter-sterilized)

**Storage:** store at 4°C to -20°C, sustain to thaw-frozen cycle. Stable for 12 months.

### 1. What is Lentivirus Concentrator?

There are a few methods to concentrate and purify crude lentivirus for high-titer, cleaner lentivirus, such as ultra-centrifugation, affinity column purification, and chemical precipitation. Each has its advantages and disadvantages. The easier method is the Polymeric precipitation without the tedious and time-consuming ultra-centrifugation process. High-titer lentivirus is desired for some Hard-to-transduced cell types, and for many *in vivo* applications.

Polymeric precipitation reagents allow for the precipitation of nanosized virus particle at low speed centrifugation. It breaks down the charges around viral particles and bring particles together.

Gentarget developed a virus concentration kit. Virus (lentivirus, retrovirus, baculoviruses, or phages) is concentrated using the non-cell toxic PEG (Poly-Ethylene Glycol) precipitation method. The concentrated virus can be used directly for in vitro and in vivo application. The trace amount of PEG does not affect the uptake of the target cells, yet can promote membrane fusion under certain conditions.

The process is easily scaled up to accommodate larger supernatant volumes, increases the lentivirus titer (IFU/ml) by 10 to 100 folds, with recovery 50% to 90%.

### 2. Lentivirus concentration protocol

The provided Pellet-Down solution is formulated PEG solution, provided as 4x stock. The virus resuspension solution is the PBS solution with stabilizer. The



following protocol provides an easy, effective procedure to concentrate lentiviral particles.

- 1) Harvest crude virus supernatants, briefly centrifuge (**3000g x 15 min** for lentivirus, or 16,000g x 15 min for phage). (alternative, filter through 0.45 um filter using the PES (polyethersulfone) filter, do not use nitrocellulose filter.
- 2) Transfer clarified supernatant to a sterile container and combine **1 volume** of Pellet-Down solution with **3 volumes** of clarified supernatant (note: this can be easily scale up according to the supernatant volume). Mix by gentle inversion.

**Note:** Volume of Pellet-Down solution to add = (crude virus volume) / 3.

- 3) Incubate mixture at 4°C >1 hr, or for overnight for convenience.
- 4) Centrifuge sample at **3000 x g for 30 minutes** at 4°C. The viral particles pellet is visible as white pellet at bottom of the tube.

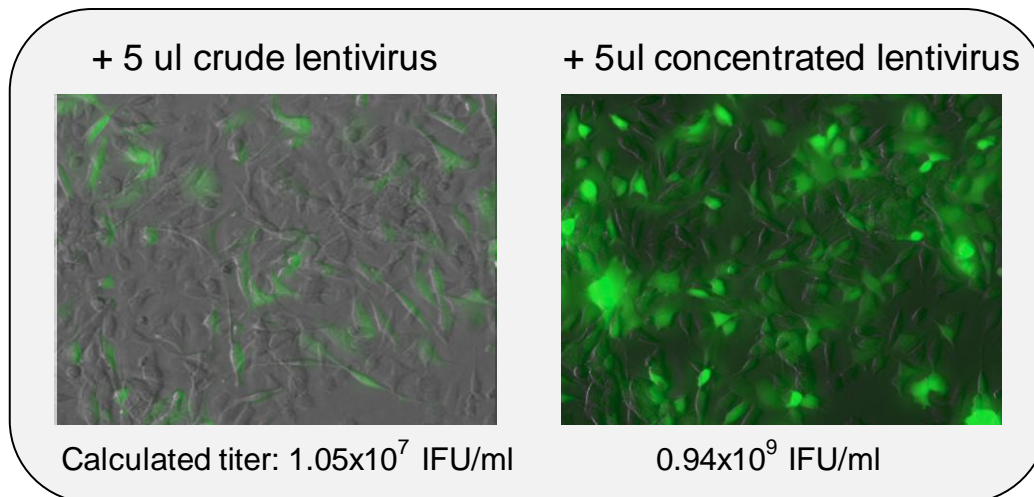
Notes:

- The pellet size is not necessarily well correlated with the virus yield. Actually, most of the pellet is the proteins serum spun down from the media.

- 5) Carefully remove all supernatant without disturb the pellet. Then, quick spin the residual, remove all traces of supernatant. The virial particles is in pellet.
- 6) Gently resuspend the pellet in 1/10 to 1/100th of the original volume using the provided virus Suspension solution.
- 7) Store the concentrated lentivirus (make single-use aliquots if desired) at <-75°C



### 3. **Example:** Lentivirus concentrated results,



**Figure 1: Lentivirus titer increase by 100 folds using Pellet-Down concentration kit.** 75 ml lentivirus supernatant (CAT#: [LVP020](#)) was mixed with 25ml of Pellet-Down solution, and resuspended into 750 ul of resuspension solution. 5 ul of each virus was added into HEK293 cells. Images taken at 48 hours post transduction. Virus titer was quantified by positive GFP cell % via EVOS Fluorescent microscope software.

### **Technical support:**

For any questions, please visit our website at <https://www.gentarget.com/contact/> or email us to [Support@gentarget.com](mailto:Support@gentarget.com)

### **Warranty and Terms:**

- 1) Gentarget warrants that the Product meets the specifications described in this manual. If the Product fails to meet these specifications, Gentarget will replace the Product or provide the purchaser with a refund upon the verification of the product failure by Gentarget. Such replacement or refund must be submitted to Gentarget within 30 days of receipt of the product. The S/H is not refundable. This limited warranty shall not extend to anyone other than the original purchaser of the Product.
- 2) This product is intended for cellular research use only, not to be used for animal in vivo applications.

**Attachment:** GenTarget's pre-made lentivirus product categories.



<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
<a href="#">Pathway Reporter</a>	Repoter Lentivirus for all kinds of pathway screening assays
<a href="#">Cell Immortalization</a>	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.
<a href="#">ImmunoOncology Research</a>	Lentivirus products for immuno therapy research: CAR and TCR; Assay Cell Lines for T-cell targeted killing assay and other cell-based assays; over-expression lentivirus products for the immune response targets; Cell surface antigens (CDs); immune checkpoint / Receptors; CRISPR gene Repair and knock-IN lentivirus; CRISPR knockout lentivirus;
<a href="#">CAR-T, TCR Lentivirus</a>	<b>CARs</b> Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; <b>TCRs</b> : MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRαV3-F2A-TRβV5-6;
<a href="#">CRISPR Gene Editing</a>	Preamde lentivirus express humanized wild-type <b>Cas9</b> endonuclease, the <b>dCas9</b> , gRNAs, <b>CRISPR</b> gene editing research
<a href="#">Epigenomic: CRISPRi and CRISPRa</a>	" <b>dCas9-Protein</b> " fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).
<a href="#">Cell-Specific Reporter</a>	a set of reporter lentiviruses to express a luminescence or fluorescent reporter (firefly Luciferase, Renilla luciferase, RFP or GFP fluorescent marker) under a tissue specific promoter
<a href="#">Infectious Antigens</a>	Lentivirus that express all kinds of infectious antigens with C-term 6His-tag.
<a href="#">Virus Like Particles (VLP)</a>	Lentiviral Like Particles, pseudo-typed with a different envelope proteins.
<a href="#">Non-integrating LV</a>	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.
<a href="#">shRNA Knockdown</a>	Knockdown verified and customized shRNA lentivirus for target knockdown,
<a href="#">microRNA lentivirus</a>	Premade lentivirus expression human or mouse <b>precursor miRNA</b> . And <b>anti-miRNA</b> lentivector and virus for human and mouse miRNA.



<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
<a href="#">Anti-miNA lentivirus</a>	Pre-made lentivirus expression a specific anti-miRNA cassette.
<a href="#">Human and mouse ORFs</a>	Premade lentivirus expressin a <b>human, mouse or rat</b> gene with RFP-Blastididin fusion dual markers.
<a href="#">Luciferase expression</a>	Premade lentivirus for all kinds of luciferase protein expression: <b>firefly and Renilla, Red-Luc and more</b> , with different antibiotic selection markers.
<a href="#">Fluorescent Markers</a>	Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, niRFP, unstable GFP and others.
<a href="#">Luminescent Imaging</a>	Lentivirus express Nano-Latern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms
<a href="#">Sub-cellular Imaging</a>	Lentivirus contain a well-defined organelle targeting signal fusioned to a fluorescent protein, great tools for live-cell imaging and for dynamic investigation of sub-cellular signal pathways.
<a href="#">Cytoskeleton Imaging</a>	A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure
<a href="#">Unstable GFP</a>	Lentivirus express the the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway assay and in knockdown / knockout detection
<a href="#">near-infrared RFP</a>	The near-infrared Red fluorescent (niRFP) expression Lentiviurs provides the whole-body images with better contrast and brighter images
<a href="#">Fluorescent-ORF fusion</a>	Pre-made lentivirus expression a " <b>GFP/RFP/CFP-ORF</b> " fusion target.
<a href="#">CRE recombinase</a>	Premade lentivirus for expressing <b>nuclear permeant CRE</b> recombinase with different flurescent and antibiotic markers.
<a href="#">CRE, Flp ColorSwich</a>	Lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" or "FRT-GFP-Stop-FRT-RFP" cassette, used to monitor the CRE or Flp recombination event in vivo.
<a href="#">SEAP Reporter</a>	lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter),



<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
<a href="#">TetR Repressor</a>	Premade lentivirus expressing TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
<a href="#">rtTA Expression</a>	rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF inducible system.
<a href="#">iPS factors</a>	Premade lentivirus for human and mouse iPS ( <b>Myc, NANOG, OCT4, SOX2, FLK4</b> ) factors with different fluorescent and antibiotic markers
<a href="#">LacZ expression</a>	Express different full length <b><math>\beta</math>-galactosidase (lacZ)</b> with different selection markers
<a href="#">Negative control lentiviruses</a>	Premade <b>negative control lentivirus with different markers</b> : serves as the negative control of lentivirus treatment, for validation of the specificity of any lentivirus target expression effects.
<a href="#">Other Enzyme expression</a>	Ready-to-use lentivirus, expressing a specific enzyme with different selection markers.
<a href="#">Ultra titer lentivirus</a>	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.