GenTarget Inc
7930 Arjons Drive, Suite B
San Diego, CA 92126
Phone: 858-6788683
Fax: 800-3804198
Email: Orders@gentarget.com

FAQ about pre-made lentiviral particles

1. What are GenTarget's Pre-made Lentiviral Particles and Lentivectors?

Lentiviral particles (LP) are lentivirus supernatant generated from lentiviral vectors (LVs) express a specific gene or RNAi construction.

Lentivectors are HIV-1 (Human Immunodeficiency Virus 1) derived plasmids. They generate a replication-incompetent lentivirus that can be transduced into almost all types of mammalian cells, including primary and non-dividing cells; they are used to deliver expression or knockdown.

GenTarget's pre-made over-expression lentivirus and knockdown shRNA lentivirus are generated from its proprietary **SureTiter™ lentiviral vector system**, our **Optional Inducible suCMV vector** and its **shRNA expression lentivectors**. Selected genes (or designed knockdown shRNA sequences) were first cloned into a lentivector, and then the lentivector was co-transfected into a 293T cell (cat# **TLV-C**) with GenTarget's proprietary packaging mix (Cat# **HT-pack**). The generated, VSV-G pseudotyped lentivirus are replication incompetent.

2. What kinds of premade lentiviral particle does GenTarget provide?

GenTarget provides ready-to-use particles for shRNA expression and gene expression. Each particles contains a sequence fully verified shRNA or a specific gene target. Particles provided **in either DMEM medium containing 10% FBS**, or **in PBS solution**. Purified viruses in PBS are provided as in vivo ready status, suitable for in vivo applications, or for suspension cell transduction, and for transduction in cell lines requiring a serum-free culture conditions.

GenTarget provides particles containing different antibiotic markers, including: Blasticidin (Bsd), puromycin (Puro), luciferase (Luc), neomycin (Neo), and fusion dual markers as: Bsd-GFP, Bsd-RFP, Puro-GFP, Puro-RFP and others.

All GenTarget's Lentiviral particles can be used for constitutive target (or shRNA) over-expression. Optionally, the same particles can be used as tetracycline inducible expression when a tetracycline regulator (tetR) protein is present (called **optional inducible expression**).

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To select the GenTarget's lentivirus products:

Product Selection Guideline for GenTarget's lentivirus:				
Lentivirus category	**Promoter types**	**Antibiotic marker**	**Fluorescent marker**	**Lentivirus formats**
Target expression; **Fluorescent markers;** **Common enzumes;** **Knockdown shRNA;** **microRNA;** **Anti miRNA**	**suCMV;** **Optional Tet inducible promoter;** **EF1a promoter;** **CAG promoter;** **Tissue or Pathway specific promoter;**	**Puromycin;** **Blasticidin;** **Neomycin;** **Hygromycin;** **Or No any antibiotic marker**	**GFP;** **RFP;** **CFP;** **BFP;** **YFP;** **niRFP;**	**Regular lentivirus in DMEM medium;** **Concentrated lentivirus in PBS;** **Ultra titer lentivirus in PBS;**

For search GenTarget's target over-expression lentivirus:
You can search a product in the search field by input: 1. Search by **gene name**: for example, "**NR2E3**"; 2. Search by gene **Alias names** or **gene_synonym** (alternative names): for example, "**PNR**", 3. Search by the gene's transcript **mRNA ID,** as NM_xxxxxx, for example, "**NM_014249**", Or you simply open this ***Product Manual*** for all available over-expression lentivirus for human, mouse or rat' genes.

3. Some GenTarget's particles are named "*In Vivo* ready". What it means?

Gentarget provides the premade particles in two formates: 1) as crude virus in DEME medium with 10% FBS. 2) as concentrated virus in PBS. we also name the purified virus in PBS as "*In Vivo* ready" because it has higher virus titer and does not contain serum (FBS). The virus in PBS is good for the

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hard-to-trasnduced cell type or serum sensitive cell types, and also can be used for direct injection for in vivo assay.

4. **How does optional inducible expression works?**

Constitutive expression of some genes may be toxic or unwanted, making controlled expression desirable. GenTarget's inducible lentiviral particles allow constitutive expression of a specific gene or a knockdown shRNA construct, and when needed and optionally, allow tetracycline-inducible expression.

Optional inducible expression is possible because the lentiviral particles contain a target gene of interest (GOI) or shRNA construct expressed under **a tetracycline regulated CMV or H1 promoter** in which two copies of the tetracycline (tet) operator sequence (TetO) has been integrated. Unlike other tetracycline inducible systems such as the Tet-On/Off system, this promoter modification does not prevent constitutive expression. In the presence of the tet repressor protein (TetR), however, transcription is repressed by the binding of TetR to the promoter. Once tetracycline or Doxycycline (Dox, a derivative of tetracycline) is added, it will bind the TetR protein and TetR will be released from the promoter, allowing transcription to begin (see more details here). This optional inducible system is tetracycline dose-dependent. The commonly used final tetracycline concentration is 1.0 µg/ml. The expression induction folds can be up to 1000 folds depends on the basal expression level.

The presence of tetR can be achieved by the following methods:

- **TetR stable cell lines** that constitutively express the TetR protein
- **Transfection** of a TetR expression plasmid and a target-inducible expression vector
- **Transduction** of the premade TetR expression lentiviral particles

Note: GenTarget **provides pre-made TetR lentiviral particles** containing different antibiotic markers that can be paired with expression particles for double selection (for generation of inducible expression system).

5. **Why use GenTarget's Pre-made lentiviral particles?**

In contrast to retroviruses, lentiviruses are imported much more actively into the nuclei of non-dividing cells and are stably integrated into the host cells' genome independent of cell cycle. Although adenoviruses are also able to transduce non-dividing cells, they are used only for transient expression

because they cannot integrate into the host cell genome. Adeno-associated virus (AAV) as well as retroviruses are able to integrate into the host genome; however, HIV-based lentiviruses are much less cell toxic, less immunogenicity, and have better transduction efficiency in many cell types, and therefore hold unique promise as gene transfer agents. (In most cases, you can use lentivirus as much as the assay allowed, but you cannot do so for Adenovirus or AAV virus because of virus' cell toxicity).

GenTarget's Pre-made Lentiviral Particles provide a ready-to-use, easy delivery method for specific target and shRNA expression, free of the often troublesome lentivector contraction and lentiviral virus production.

GenTarget engineered transfer and packaging lentivectors offer the highest lentiviral titers available along with a wide range of selection features including **a broad variety of antibiotic selection markers and fluorescent-antibiotic fusion markers for real-time transduction monitoring.** Additionally, the same lentiviral particles can be used for either constitutive or tetracycline-inducible expression as needed. Concentrated Particles are provided in PBS for in vivo application, or serum sensitive culture.

Applications for Pre-made Lentivirus:

- Delivery of expression into hard-to-transfect cell types, such as neuronal cells, without using any transfection reagents
- Highly reproducible and controllable expression delivery method
- Creation of stable cell lines for long-term, high-level expression
- Expression of genes in primary or drug-arrested cells
- Creation of transgenic animals
- Sub-cellular localization analysis by organelle targeting

6. **How was the titer measured for GenTarget's pre-made lentivirus?**

The titer of each lot of pre-made lentiviral particles is measured by fluorescent cell counting using either Guava cell sorting or a microscope. Each fluorescence positive cell is counted as one Infection Function Unit (IFU); total positive fluorescent cells are calculated based on the percentage of fluorescent cells and total cell number at the time of transduction. The final titer is the total IFUs in 1ml of virus stock. For non-fluorescent particles, the titers were measured by ELISA for P24 protein levels, as ng/ml. The P24 value may be converted into TU or IU or IFU units. However, different cell types have different conversion ratios, and P24 values may not be consistent with true IFU units. In general, all

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titers (IFU, TU or IU) are used for reference, and real biological titers are cell-type dependent. GenTarget's fluorescent-labeled lentiviral particles allow quick and easy measurement of **true transduction efficiency** by visualization of the fluorescent signal.

7. What is MOI?

MOI (Multiplicity of Infection) is the average copy number of lentiviral particles per target cell genome in the infected cell population, or the number of viral particles per cell. Since not all particles can infect cells, the MOI does not directly correspond to the percentage of cells infected. MOI is calculated by counting the number of cells and the number of viral particles to be used for transduction. A higher MOI will generate more integration and as a result, a higher level of expression. To obtain optimal expression for your specific application, a range of MOIs (e.g. from 1 to 20) should be tested. Theoretically, an MOI of less than one (such as MOI=0.3) should be used to achieve single copy integration. Practically, at MOI =0.3, only 5% - 20% of the cells will be transduced, depending on the cell type, and the majority of transduced cells will only have one copy of insert. In most cases, you can simply add 50 µl of our premade LP per well in a 24-well plate and not worry about calculating MOI.

8. How to use the pre-made lentiviral particles?

GenTarget's pre-made lentiviral particle are **ready to use**. Simply add 50 µl of particles into cultured cells in a 24-well plate. Within 48 to 72 hours, you can check the viral transduction efficiency by visualizing the fluorescent signal under a microscope (Note: some cell types need more time to see the fluorescent signal, fluorescence visualization in some cell types may require up to 10 days). If the fluorescent marker is not available, you can test the target expression. Please follow the detailed protocol included in product manual.

Some additives, such as polybrene, can enhance the transduction efficiency. And many factors can affect transduction efficiency. The main factor is cell type. An actively dividing cell line gives a much higher transduction rate than a non-dividing line; therefore, if you are transducing non-dividing cells, a higher MOI should be used for optimal expression. Please refer to our **recommended transduction protocols** for more information.

(**Note**: Polybrene has been reported to enhance viral transduction. Most GenTarget's pre-made un-concentrated particles already contain 60 µg/ml of polybrene (10x stock). Please be advised, Polybrene is toxic to some cell types.)

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9. Is it safe to use GenTarget's Lentiviral vector system?

Yes. GenTarget lentiviral vectors include the most advanced bio-safety features developed for lentiviral vectors. The viral envelope (VSVG) and accessory proteins (gag-pol and rev) are separate from the expression lentivectors, minimizing the potential for homologous recombination. Additionally, those packaging components necessary for replication are excluded once the viral particles are packaged. Furthermore, our lentivectors are derived from the third generation lentiviral system which includes a 3'-LTR self-inactivation (SIN) mechanism. These features ensure that the premade particles are not replicable (replication incompetent), meaning the self-replication of the original virus is impossible.

However, Please use extra caution when using lentiviral particles**.** The CDC suggests that lentiviral particles be treated as Bio-safety Level 2 organisms, and although these lentiviral vectors will generate only replication-incompetent lentivirus, a Bio-safety Level 2 (BSL-2) facility is required. Remember, you are working with transduction particles that can infection human cells. **Wear gloves at all times when handling lentiviral particles!** Please refer to CDC and NIH websites (see references) for more details regarding safety issues.

***These products are for research use only, not for therapeutic, clinical, or other uses*.**

10. **How stable are the pre-made lentiviral particles?**

Pre-made lentivirus are stable for at least one year if they are kept at -80 ^{o}C at all times until used. (Lentivirus is shipped in dry-ice package). Repeated freeze-thaw cycles should be avoided since virus titer decreases at ~ 5% to 10% per cycle. Re-freeze unused particles, or keep them at -4 ^{o}C for re-use within 3-5 days.

11. **Can I use pre-made particles to generate the stable cell line?**

Yes, you can use these particles to generate a stable cell line. Pre-made lentiviral particles contain different selection markers, allowing antibiotic selection following transduction. GenTarget also provide **pre-made cell lines** for some commonly used targets, and we offer a **stable cell line creation service** for generating a stable cell line expressing your specific target in the cell type of your choice. This service provides a fast turnaround time, and lower costs compared to other providers. Please contact us for a quote.

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To make a constitutively expressing stable cell line, the target must be integrated into the host cells' genome. Random integration (such as by plasmid transfection) demonstrates large variability of expression depending upon the transcription levels at the integration sites. Also, random integration often takes the form of independent integration of the target and the selection marker, requiring large-scale screening for selection of positive clones. Generally, less than 10% of resistant clones express the transgene. In contrast, lentivirus preferentially integrates the full viral genome into active transcription sites or "hot-spots". Consequently, most resistant clones demonstrate transgene expression which makes the colony selection much easier.

12. **What are the advantages for using lentivirus to generate stable cells?**

Compared to conventional stable cell line construction, lentivirus exhibits a much higher positive clone rate, with the target always co-existing with the selection marker. The cost, labor, and time are substantially lower than transfection based stable cell line generation.

References:

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2. Zufferey, R., et al., Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. J Virol. 72, 9873-80 (1998).
3. Tom Dull, et al., A third-generation lentivirus vector with a conditional packaging system. J Virol. 72. 8463-8471 (1998)
4. Mukesh Kumar, et al., Large-Scale production of pseudotyped lentiviral vectors using baculovirus GP64. Human gene therapy. 14:67-77 (2003).
5. MOLECULAR THERAPY Vol. 14, No. 4, October 2006.
6. Robert Mcknight, et al., Matrix-attachement ragions can impart position-independent regulation of a tissue-specific gene in transgenic mice. P.N.A.S. 89:6943-6947, (1992).
7. NIH Guidelines for Biosafety Considerations for Research with Lentiviral Vectors. (Link).
8. CDC guidelines for Lab Biosafety levels (Link).

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Attachment: GenTarget's Pre-made lentivirus Products:

Product Category	Product Description (please click category name to see product's pages)
Human and mouse ORFs	Premade lentivirus expressin a **human, mouse or rat** gene with RFP-Blastididin fusion dual markers.

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Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, **GFP / RFP/ CFP/ BFP / YFP**.
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression**: firefly and Renilla** with different antibiotic selection markers.
CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers.
LoxP ColorSwitch	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
CRISPR /hu CAS9	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease for genomic editing with **CRISPR**
TetR inducible expression repressor	Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
Pathway specific report lentivirus	Premade lentivirus for monitoring a speific signal pathway or a tissue speicific promoter strength, also used for generation of deisred cell lines for cell based assays.
iPS factors	Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibitoic markers
T-antigen Expression	Express different large and small T antigen with different selection markers
Cell Organelle imaging	Premade lentivirus for cell organelle imaging. The fluorescent marker **GFP/RFP/CFP was sub-cellular localized** in different cell organelle for living cell imaging.
LacZ expression	Express different full length **β- galactosidase (lacZ)** with different selection markers
Anti-miNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.
Fluorescent-ORF fusion	Pre-made lentivirus expression a "**GFP/RFP/CFP-ORF**" fusion target.
Pre-made shRNA lentivirus	Premade shRNA lentivirus for knockdown a specific genes (**P53, LacZ, Luciferase** and more).
microRNA and anti-microRNA lentivirus	Premade lentivirus expression human or mouse **precursor miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA.
Negative control	Premade **negative control lentivirus with different markers**: serves as the negative control of lentivurs treatment, for

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lentiviruses	validation of the specificity of any lentivirus target expression effects.
Other Enzyme expression	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.

