



## FAQ about pre-made lentiviral particles

### 1. What is Gentarget's pre-made lentiviral particles?

Lentiviral particles (LP) are lentivirus supernatant generated from lentiviral vectors (LV) that contains 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 kinds of mammalian cell types, including primary cells and non-dividing cells, and used to deliver the expression or knockdown in mammalian cells.

Gentarget pre-made lentiviral particles are generated from its proprietary [SureTiter™ lentiviral vector](#) system, its [tetracycline inducible suCMV vector](#) or its [inducible shRNA expression lentivectors](#). Selected genes (or designed knockdown shRNA sequences) were first cloned into lentivector. Then, the sequence verified lentivector was co-transfected with Gentarget's proprietary packaging mix (Cat# **HT-pack**) into a 293T cell (cat# **TLV-C**). The VSV-G pseudotyped virus was packaged in DMEM medium with 10% heat-inactivated FBS or in serum-free medium. For in vivo ready lentivirus, the particles were purified and buffer changed into PBS solution. Generated lentiviral particles were provided in 200ul/vial at  $> 1 \times 10^{7-9}$  IFU/ml.

### 2. What kind of premade particles provided by Gentarget?

Gentarget provides ready-to-use particles for shRNA expression and gene expression. Each particles contains sequence fully verified shRNA or specific gene target. Particles provided **in either DMEM medium containing 10% FBS, in serum-free medium or in PBS solution**. The **serum-free viruses** contain no human or animal origin components, best suitable for any serum-sensitive cultures. **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), Bsd-GFP, Bsd-RFP, Puro-GFP, Puro-RFP and others.**



**All Gentarget's Lentiviral particles can be used for constitutive expression of shRNA or specific gene.** Optionally, the same particles can be used as tetracycline inducible expression when a tetracycline regulator (tetR) protein is present (see next section for details).

### 3. **How tetracycline inducible particle works?**

Constitutive expression of some human gene may be toxic or unwanted. Therefore, a controlled expression is desirable. Gentarget provided inducible lentiviral particles to satisfy this need. Most Gentarget's premade lentivirus constitutively express a specific human or mouse gene, or a knockdown shRNA construct. However, when inducible is desired, the particles can be used as tetracycline induced expression.

Those particles contains sequence fully verified genes or shRNA, expressed under **a tetracycline regulated CMV promoter or H1 promoter** in which two copies of tetracycline (tet) operator sequences was integrated. Unlike other tetracycline inducible system (such as Tet-On/Off system), this promoter modification does not change the promoter's constitutive expression status. The GOI or shRNA can be expressed without any induction. However, in the presence of a repressor protein (tetR), the transcription of CMV or H1 was repressed by the binding of tetR to the promoter. Once the tet or Doxycycline (Dox), a derivative of tetracycline is added, the tetR protein switch binding to tet and released from promoter, and the transcription started (please see our [weblink](#) for more details). **So it is an optional inducible system.** The induced expression could be tetracycline dose dependent. The common used concentration is 1ug/ml of tetracycline.

The repressor protein (tetR) must be present in order to use the particles as tet induction system. The presence of tetR can be achieved by the following methods:

- Expression particles are used in a tetR expression stable cell line that constantly express tetR protein;
- Transfect a tetR expression plasmid before transduce lentiviral expression particles;
- Co-transduce both the tetR repressor particles and the expression particles into the sample cell line;



**Note:** Gentarget Inc [provides premade tetR lentiviral particles](#) containing different antibiotic markers that can be paired with expression particles for double selection.

#### 4. **Why use Gentarget's Pre-made lentiviral particles?**

Unlike traditional retroviral system, Lentivirus is much more actively imported into the nuclei of non-dividing cells and stably integrated into the host cell's genome independent of cell cycle. Although adenovirus is also able to transduce non-dividing cells, it is only for transient expression because it cannot integrate into host cell's genome. Thus lentivirus holds unique promise as gene transfer agents.

Gentarget's Pre-made Lentiviral particles provide a ready-to-use delivery method for a specific target or shRNA without worry about the often troublesome lentiviral virus production process.

With its engineered transfer and packaging vectors, Gentarget's pre-made particles demonstrated the highest lentiviral titers and with wide range of selection features (**different antibiotic markers and realtime transduction monitoring with fluorescent fusion markers**). The particles can be used as constitutive expression or as tetracycline inducible expression. It also provides a realtime transduction monitoring method by visualizing the embedded fluorescent signal under microscope.

#### **The main applications for pre-made LP are:**

- ✿ Deliver gene or shRNA into hard-to-transfected cell types (such as neuron cells), and no need any transfection reagents;
- ✿ Deliver gene or shRNA in highly reproducible and controllable methods by using more or less the lentivirus;
- ✿ Can be used for constitutive high expression or as tetracycline inducible expression.
- ✿ Creating stable cell lines for long-term high level expression in your own cell line, in a cost and labor effective way;
- ✿ Expressing genes or RNAi in primary cells, or drug-arrested cells.
- ✿ It is also great too for making transgenic animals.
- ✿ Organelle targeted lentivirus provides great tools for sub-cellular localization analysis.



## 5. **How was the titer measured for Gentarget's pre-made LP?**

Virus titers of pre-made lentiviral particles were tested at lot to lot basis by fluorescent cell counting (%) (via Guava cell sorting or under microscope). Each positive fluorescent cell was counted as one unit of IFU (Infection Function Unit). The total positive fluorescent cells were calculated based upon the percentage of fluorescent cells and total cell numbers at the time of transduction. The final titer was calculated as the total IFU at 1ml of virus stock. (**Note:** for non-fluorescent particles, the titers were measured by ELISA P24 assay, showed as P24 protein value as: ng/ml. You may convert the P24 value into TU or IU, however, different cell types have different converting rate. And P24 value may not consistent with its true IFU units. So in general, all titers (listed as IFU, TU or IU) are used as reference. The real biological titers are cell type dependent.). Gentarget provided fluorescent labeled LP demonstrates the **true transduction efficiency** by visualized the fluorescent signal, which provides an easy, quick check for virus performance.

## 6. **How to use the pre-made lentiviral particles?**

Pre-made lentiviral particle are in Ready to use status. **Simply add 5ul to 50ul** of LP into cultured cells in 24-well plate. At 72 hours later, you can check virus' transduction efficiency by visualizing fluorescent signal under microscope (Note: some cell types need longer time to see the fluorescent signal, up to 10 days). For details about how to use it, Please follow the protocols in product manual.

(**Note:** Polybrene was reported to enhance virus transduction. Some of our pre-made LP is provided as 10x stock containing ~ 60 ug/ul of polybrene. But no polybrene was added for our serum-free particles. Be noticed that polybrene is toxic to some cell types.)

## 7. **Is it safe to use Gentarget's Lentiviral vector system?**

Yes. Gentarget lentiviral vectors have adapted most advanced bio-safety features in lentiviral vector development. All viral coding sequences were not included in our lentivectors. The viral envelope (VSVG) and accessory proteins (gag-pol and rev) are separated from the expression lentivectors, which minimize the potential for homologous recombination. And those necessary packaging components (for replication) are excluded once viral particle are packaged. These features ensure that the regeneration of the starting virus is impossible. Furthermore, our lentivectors are the 3<sup>rd</sup> generation lentiviral system with the 3'-LTR self-inactivation mechanism. **It will only generate replication-incompetent lentivirus.** However, the CDC suggests that Lentiviral particles should be treated as Bio-safety



Level 2 organisms. Thus a Bio-safety Level 2 (BSL-2) facility is required. Please use extra caution when using lentiviral particles. Remember, you are dealing with transduction particles which can infection human cells. **Wear glove all the time at handling Lentiviral particles!** Please refer to CDC and NIH's links (see references) for more details regarding to safety issues. Those products are for research use only, not for therapeutic, clinical or other uses.

## 8. **How much LP should I use? What's MOI? Should I care about it?**

Many factors can affect transduction efficiency. Not all Viral particles floating in culture medium can finally transduce (or infect) into the cells. Some additives can enhance the transduction efficiency such as polybrene. But cell type is the main factor to determine the transduction efficiency. An actively dividing cell line give much higher transduction rate than non-dividing cell types. If you transduced non-dividing cells, more MOI has to be used for your optimal expression. Please refer to our [recommended transduction protocols](#) as general reference.

MOI (Multiplicity of Infection) is the average copy number of lentiviral particles per genome of target cell in the infected cell population. Since not all particles can be infected into cells, the MOI does not directly correspond to percentage of infected cells. MOI is calculated by counting the number of the cells and the number of the viral particles to be transduced. The number of viral particles per cell is defined as multiplicity of infection (MOI). More MOI generates more integration and as a result, higher level of expression. To obtain optimal expression for your specific application, a range of MOIs (e.g. from 0.1 to 20) should be tested. For example, to achieve single copy integration, theoretically, MOI has to be used at less than one (such as MOI=0.3). Practically, at MOI =0.3, only 5% ~ 20% cell will be transduced dependent upon the cell types, and majority transduced cells should only have one copy of insert. For most cases, you may simply add 50ul of our premade LP into one well in 24-well plate without worry too much about MOI.

## 9. **How stable is the pre-made lentiviral particles?**

Pre-made LP should keep at -80°C all the time until use. It is stable for at least one year. Repeat thaw-freeze cycles should be avoided since virus titer decreases at ~ 5% to 10% from each cycle. You can re-freeze unused (leftover) LP, or keep it at -40°C if you will re-use it in short time. Pre-made LP is stable for about 3-5 days at 4°C.



## 10. **Can I use pre-made LP to generate the stable cell line? What are the advantages for using lentivirus to generate stable cells?**

Yes. Pre-made lentiviral particles contain different selection markers. So you can use antibiotics to select the resistant colonies after transduction. Gentarget provide [pre-made cell lines](#) for some common used targets. And we also provide [stable cell line creation service](#) for generating the stable cell line with your specific target in your desired cell types, at much fast turnaround time, lower costs compared to other providers. Please contact us for a quote.

To make stable cell line, target has to be integrated into host cells' genome for a stable, constitutive expression. Randomly integration (such as by plasmid transfection) demonstrates large variety in expression dependent upon the transcription levels at integration sites. And random integration often causes the independent integration between the target and the selection marker, which requires the large screening scale for selecting the positive clones (resistant with high expression). In general, less than 10% resistant clones express the transgene. In contrast, lentivirus preferentially integrates into transcription active sites (hot-spots) with a full virus genome [5]. So most resistant clones demonstrate the trans-gene expression. Besides, engineered lentiviral transfer vector that embedded a matrix-attachment region (MARs) sequence may provide position-independent transgene expressions. Compared to conventionally stable cell line construction, lentivirus has much higher positive clone rate and target is always co-exist with the selection marker, thus substantially reduces the cost, labor and time in selection of high-level expression stable clones.

### References

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