



## 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 then be transduced into virtually all kinds of mammalian cell types, including primary cells and non-dividing cells, and thus used to deliver the expression or knockdown in mammalian cells.

Gentarget pre-made lentiviral particles are generated from its proprietary [SureTiter™ lentiviral vector](#) system or in its [tetracycline inducible suCMV vector](#) or its [inducible shRNA expression lentivectors](#). Selected genes were first cloned into lentivector. Then, after sequencing verification of the genes, the lentivectors were co-transfected with Gentarget's proprietary packaging mix (Cat# [HT-pack](#)) into a 293T cell (cat# [TLV-C](#)). The VSV-G pseudotyped virus was packed in DMEM medium with 10% heat-inactivated FBS or in serum-free medium. Generated lentiviral particles were provided in 200ul/vial at  $> 1 \times 10^7$  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 or in serum-free medium without any human or animal origin components. **Serum-free particles** are best suitable for suspension cell transduction or for the cell lines requiring a serum-free culture conditions. Some particles were concentrated and buffer exchanged into PBS, and provided as *In Vivo* ready concentrated status.

Gentarget's LP can be used for constitutive high expression of shRNA or specific gene. And the same particles can be used as tetracycline inducible expression when a tetracycline regulator (tetR) protein is present.

### 3. How tetracycline inducible particle works?

Constitutive expression of some human gene may be toxic or unwanted. Therefore, a controlled expression is desirable. Gentarget generated inducible ready-to-use lentiviral particles to satisfy this need. Those



particles contains sequence fully verified genes, expressed under a tetracycline regulated suCMV promoter in which two copies of tetracycline (tet) operator sequences was integrated. In the presence of a repressor protein (tetR), the transcription of suCMV was repressed by the binding of tetR to the tet operator sequences. 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). The induced expression could be tetracycline dose dependent, a common used concentration is 1ug/ml of tetracycline.

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

- Particles are used in a tetR expression stable cell line that constantly express tetR protein;
- Transfect a tetR expression plasmid before transduce lentiviral particles;
- Co-transduce both the tetR repressor particles and the gene expression virus into the sample cell line (applied with equal MOI);

#### 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 LP provides a ready-to-use delivery method for a specific target without worry about the often troublesome lentiviral virus production process. With its engineered transfer and packaging vectors, Gentarget's pre-made LP demonstrated the highest lentiviral titers and highest target expression. The particles can be used as constitutive expression or as tetracycline inducible expression. It also provides a realtime titer / **performance monitoring method** for LP by visualizing the RFP 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.

## 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 RFP fluorescent signal under microscope (with a red light filter, Ex ~545nm/Em ~620). For details about how to use it, Please follow the protocols in product manual.

(**Note:** Polybrene was reported to enhance virus transduction. The pre-made LP is provided as 10x stock containing ~ 60ng/ul of polybrene. For serum-free particles, you may add polybrene if desired. Be noticed that polybrene is toxic to some cell types.)

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

Yes. Gentarget SureTiter lentiviral vectors have adapted all biosafety features for lentiviral vector development. It is the 3<sup>rd</sup> generation lentiviral system with 3'-LTR self-inactivation mechanism. **It will only generate replication-incompetent lentivirus.** However, the CDC suggests that Lentiviral particles should be treated as Biosafety Level 2 organisms. thus a Biosafety 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. **Ware glove**



**all the time at handling Lentiviral particles!** Please refer CDC and NIH's links (see references) for more details regarding to safety issues. Those products are for research use only, not for therapeutical, drug 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 transducer (or infect) 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. Gentarget's pre-made lentiviral particles are provide as 10x stock. So simply add viral stock at 1:10 ratio of culture medium with adjusted cell numbers to obtain the desired MOIs. Please refer to our recommend transduction protocol in each product manual.

For transduction MOI: calculate 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.

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

Pre-made LP should keep at -80°C all the time before use and 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 1 week at 4°C.

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



Yes. Pre-made lentiviral particles contain a Blasticidin selection marker. So you can use blasticidin to select the resistant colonies after transduction.

GenTarget provide [pre-made cell lines](#) for some common 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 cost 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 (that such as by transfection) demonstrates large variety expression dependent upon the transcription levels at integration sites. And random integration often resulted in the independent integration between the target and the selection marker, which lead to large scale of screening works for selecting the positive clone (resistant with high expression). In general, less than 10% resistant clones express the transgene. In contrast, lentivirus transduction has a tendency to integrate in high transcription level sites (hot-spots) with a full virus genome. 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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7. [CDC guidelines for Lab Biosafety levels \(Link\)](#).