

## [FAQ about pre-made lentiviral particles](#)

### 1. What are pre-made lentiviral particles?

Lentiviral particles (LP or LVP) are lentivirus supernatants generated from lentiviral vectors (LV) that contain a specific gene or RNAi construct. Lentivectors are HIV-1 (Human Immunodeficiency Virus 1) derived plasmids that generate a replication-incompetent lentivirus which can be transduced into virtually any mammalian cell type including primary cells and non-dividing cells.

Pre-made lentiviral particles are generated with the proprietary SureTitre™ lentiviral vector system, tetracycline inducible suCMV vector system or the inducible shRNA expression lentivectors. Selected genes were first cloned into the lentivector, gene sequence verified and co-transfected with proprietary packaging mix (Cat# [HT-pack](#)) into a 293T cells (cat# [TLV-C](#)). The VSV-G pseudo-typed virus was packed in DMEM culture medium with 10% heat-inactivated FBS or in serum-free medium without any human or animal origin components. The generated lentiviral particles are provided in 200µl/vials at > 1x 10<sup>7</sup> IFU/ml.

### 2. What kind of premade particles are provided by AMSBIO?

**AMSBIO** can provide [ready-to-use particles for transgene expression](#) or for shRNA expression.

**Serum-free particles** are best suited for suspension cell transduction or for cell lines requiring serum-free culture conditions. **Particles in PBS** are ideal for use *in vivo* due to having been concentrated and buffer exchanged into PBS.

**AMSBIO** lentivirus particles can be used for high constitutive expression of shRNA or specific genes. They can also be used for tetracycline inducible expression when a tetracycline regulator (tetR) protein is present (for example by transduction of a cell line stably over-expressing tetR, or by co-transduction).

### 3. How do tetracycline inducible particle work?

Constitutive expression of some human genes may be toxic or unwanted. Therefore, controlled inducible expression is desirable. **AMSBIO** has generated inducible ready-to-use lentiviral particles to solve this problem. The particles contain fully sequence

verified genes, expressed under a tetracycline regulated suCMV promoter in which two copies of tetracycline (tet) operator sequence was integrated. In the presence of a repressor protein (tetR), the transcription of suCMV is repressed by the binding of tetR to the tet operator sequences. Once tetracycline or doxycycline (Dox), a derivative of tetracycline is added, the tetR protein switches its binding to tetracycline and is released from the suCMV promote allowing transcription of the transgene or shRNA. Expression is dose dependent, but tetracycline is commonly used at a concentration of 1µg/ml.

The repressor protein (tetR) must be co-expressed in order to use the particles as tetracycline induced system. The presence of tetR can be achieved by the following methods:

- Using a cell line stably expressing tetR,
- Transfect cells with a tetR expression plasmid before transduction with lentiviral particles,
- Co-transduce both the tetR repressor particles and the gene expression virus into the sample cell line (applied with equal Multiplicity of Infection);

#### 4. Why use pre-made lentiviral particles?

Unlike a traditional retroviral system, lentivirus is much more actively imported into the nuclei of non-dividing cells and **is also stably integrated** into the host cell's genome, independent of cell cycling. Although adenovirus is also able to transduce non-dividing cells, it can only be used for transient expression and cannot integrate into host cell's genome.

Pre-made lentivirus provides a ready-to-use delivery method for a specific target without the worry and often troublesome lentivirus production process. With its engineered transfer and packaging vectors, **AMSBIO** pre-made lentivirus demonstrates the highest lentiviral titres and highest target expression. They also provide a real-time titre / performance monitoring method by visualising the co-expressed RFP fluorescent signal under a microscope.

The main applications for pre-made lentivirus are:

- Easy gene or shRNA delivery into hard to transfect cell types (such primary cells - neuron cells or drug-arrested cells)
- Highly reproducible and controllable by using more or less of the lentivirus
- Can be used for high constitutive expression or as tetracycline inducible expression
- The creation of stable cell lines for long-term high expression levels in your own cell line, in a cost and labour effective way
- The production of transgenic animals

- Organelle targeting as a tool for sub-cellular localization analysis (in development)

## 5. How is the titre measured for pre-made lentiviral particles?

All lentiviral expression plasmids co-express RFP. Virus titres of pre-made lentiviral particles are tested lot to lot by fluorescent cell counting (%) (using a Guava cell sorter or under a 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 titre was calculated as the total IFU of a 1ml virus stock.

## 6. How do you use pre-made lentiviral particles?

Pre-made lentiviral particles are provided **Ready-to-use**. Simply add 5-50 $\mu$ l of lentivirus to cells cultured in a single well of a 24-well plate (For details please see [the detailed protocols for transduction](#)). 72 hours later, you can check virus' transduction efficiency by visualising the RFP fluorescent signal under a microscope (with a red light filter, Ex  $\sim$ 545nm/Em  $\sim$ 620).

(**Note:** Polybrene has been reported to enhance virus transduction. The pre-made lentivirus contains 60ng/ $\mu$ l polybrene. For serum-free particles, you may add polybrene if desired. Polybrene is toxic to some cell types.)

## 7. Is it safe to use the lentiviral vector system?

**Yes. AMSBIO** lentiviral vectors have adopted all biosafety features for lentiviral vector development. This 3<sup>rd</sup> generation lentiviral system with a 3'-LTR self-inactivation mechanism will only generate replication-incompetent lentivirus. However, the CDC suggests that lentiviral particles should be treated as Biosafety Level 2 organisms, therefore such a facility is required. Please use extra caution when using lentiviral particles can infection human cells. Wear gloves at all times. Please refer to the CDC and NIH's links (see references) for more details regarding safety issues. Those products are for research use only and are not for therapeutic, drug or other uses.

## 8. How much lentivirus particles should I use? What is Multiplicity of Infection (MOI), should I care about it?

The multiplicity of infection or MOI is the ratio of infectious agents (e.g. lentivirus particles) to infection targets (e.g. cells).

However, many factors can affect transduction efficiency. Not all viral particles floating in culture medium can transduce (or infect) cells and not all cells can be transduced. Some additives, such as polybrene, can enhance the transduction efficiency, but the cell type is the main factor to determining transduction efficiency. An actively dividing cell line gives a much higher transduction rate than non-dividing cell types. If you transduce non-dividing cells, a higher MOI has to be used for optimal expression. Pre-made lentiviral particles are provided as 10x stock, so simply add viral stock at 1:10 ratio of culture medium with adjusted cell numbers to obtain the desired MOI. Please refer to our recommend transduction protocol in each product manual.

In general a higher MOI will generate a higher number of transductions per cell and therefore a higher number of transgene integrations, and as a result, higher 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, the MOI should be less than one (e.g. MOI=0.3). Practically, at MOI =0.3 only 5-20% of cells will be transduced (depending upon the cell type), but the majority of transduced cells should only have one copy of the transgene.

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

Pre-made lentivirus should be stored at  $-80^{\circ}\text{C}$  and are stable for at least one year. Repeated freeze-thaw cycles should be avoided since the virus titre decreases 5-10% with each cycle. You can re-freeze unused/leftover lentivirus, or store it at  $-4^{\circ}\text{C}$  for approximately 1 week.

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

**Yes.** Pre-made lentiviral particles contain a Blasticidin selection marker. Therefore use blasticidin to select for resistant colonies after transduction.

**AMSBIO** provides [pre-made stable cell lines for some common targets](#), but we are also able to provide [a custom service](#) for generating a stable cell line expressing a

specific target gene in a specific cell type, at much fast turnaround time and lower cost than other providers. [Please contact us for a quote.](#)

To make a stable cell line, the gene has to be intergraded into host cells' genome for stable, constitutive expression. Random integration (for example by transfection) can generate clones with a large variety of expression levels dependent on the integration site. Random integration can also often result in the independent integration of the target transgene and the selection marker, leading to a large scale screening for positive, high expression clones. In contrast, lentivirus transduction has a tendency to integrate the transgene and full virus genome in areas characterized by high levels of transcription (hot-spots). Lentiviral transfer vectors used in our custom stable cell line generation also possess a matrix-attachment region (MARs) sequence that may provide position-independent transgene expression. Compared to conventionally stable cell line construction, those created with lentivirus have a much higher positive clone rate and the target always co-exists with the selection marker therefore substantially reducing costs, labour and time in selection of high-level expression stable clones.

#### References:

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4. Mukesh Kumar, et al., Large-Scale production of pseudotyped lentiviral vectors using baculovirus GP64. *Human gene therapy*. 14:67-77 (2003).
5. Robert Mcknight, et al., Matrix-attachment regions can impart position-independent regulation of a tissue-specific gene in transgenic mice. *P.N.A.S.* 89:6943-6947, (1992).
6. NIH Guidelines for [Biosafety Considerations for Research with Lentiviral Vectors](#). (Link).
7. [CDC guidelines for Lab Biosafety levels](#) (Link).