

Ready-to-use Lentiviral Particles for β -Lactamase expression

Cat#	Product Name	Amounts
LVP335-GB	β -Lactamase (GFP-Bsd) lentiviral particles	1x10 ⁷ IFU/ml x 200ul
LVP335-GB-PBS	β -Lactamase (GFP-Bsd) lentiviral particles, in vivo ready	5x10 ⁸ IFU/ml x 200ul
LVP335-RB	β -Lactamase (RFP-Bsd) lentiviral particles	1x10 ⁷ IFU/ml x 200ul
LVP335-RB-PBS	β -Lactamase (RFP-Bsd) lentiviral particles, in vivo ready	5x10 ⁸ IFU/ml x 200ul
LVP335-Neo	β -Lactamase (Neo) lentiviral particles	1x10 ⁷ IFU/ml x 200ul
LVP335-Neo-PBS	β -Lactamase (Neo) lentiviral particles, in vivo ready	5x10 ⁸ IFU/ml x 200ul
LVP335-Bsd	β -Lactamase (Bsd) lentiviral particles	1x10 ⁷ IFU/ml x 200ul
LVP335-Bsd-PBS	β -Lactamase (Bsd) lentiviral particles, in vivo ready	5x10 ⁸ IFU/ml x 200ul
LVP335-Puro	β -Lactamase (Puro) lentiviral particles	1x10 ⁷ IFU/ml x 200ul
LVP335-Puro-PBS	β -Lactamase (Puro) lentiviral particles, in vivo ready	5x10 ⁸ IFU/ml x 200ul
LVP335-GP	β -Lactamase (GFP-Puro) lentiviral particles	1x10 ⁷ IFU/ml x 200ul
LVP335-RP	β -Lactamase (RFP-Puro) lentiviral particles	1x10 ⁷ IFU/ml x 200ul
LVP335-luc	β -Lactamase (Luciferase) lentiviral particles	1x10 ⁷ IFU/ml x 200ul

Storage: <-70 °C, avoid repeat freeze/thaw cycles. Stable for >6 months.

Product Description:

Lentiviral system is a gene delivery tool using lentivectors for gene expression or knockdown. Lentivectors are HIV-1 (Human Immunodeficiency Virus 1) derived plasmids, used to generate lentiviral particles (lentivirus) that can be transduced into virtually all kinds of mammalian cell types or organs, including stem cells, primary cells and non-dividing cells both *in vivo* and in **cell culture** system. Particles stably integrate into the transduced cells' genome for long term expression. Therefore, lentivirus holds unique promise as gene transfer agents.

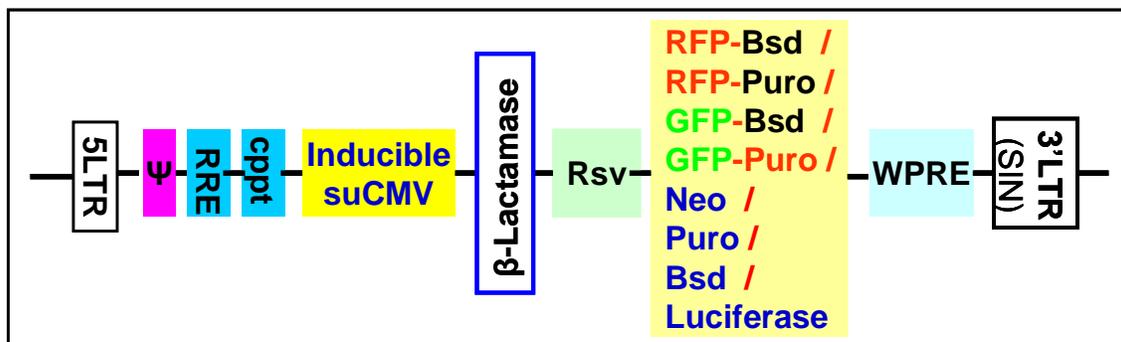
Pre-made **β -Lactamase** (type: TEM-1) lentiviral particles are generated from an optional **inducible lentiviral system** with different selection markers (see vector scheme below). **β -Lactamase** was expressed under suCMV promoter, selection marker under Rsv promoter (see vector schematic map below). VSV-G pseudotyped lentiviral particles are generated in 293T cell.

β -Lactamase are enzymes produced by some bacteria and are responsible for their resistance to beta-lactam antibiotics, like penicillins. Up to 90% of ampicillin resistance in E. coli is due to the production of TEM-1 type of β -Lactamase. It catalyzes the hydrolysis and aminolysis of depsipeptides, and is used in enzyme kinetic studies (in all kinds of β -Lactamase based colorimetric reporter assays) and other anti-microbial programs for identification of its inhibitor.

Ready-to-use Lactamase lentiviral particles are provided in two formats:

- packaged in 10% of FBS in DMEM containing 10% FBS and 60ug/ml of polybrene;
- particles were concentrated and buffer exchanged in PBS without any human or animal origin components;

For more details about premade particles, please see <http://www.amsbio.com/FAQ-Premade-Lentiviral-particles.pdf>



Key features:

1. High Lactamase expression level and high viral titer;
2. Used as constitutive expression, or optionally as tetracycline inducible expression;
3. Easy transduction monitoring via the fluorescent signal;
4. Dual markers: transduced cells can be sorted via a fluorescent signal or selected via antibiotics;
5. **The lentiviruses are ready and easy to use; simply add 50ul into your cell culture in 24-well plate.** (Note: Depending on your specific needs, you may design the transduction with different MOI for different levels of expression.)

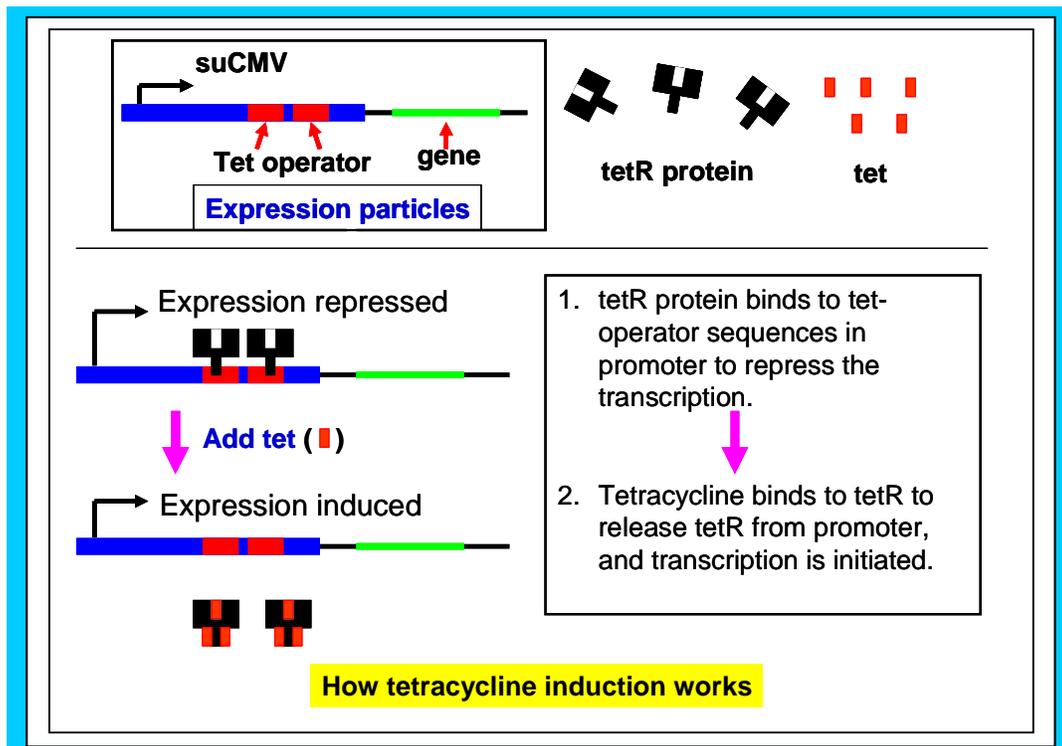
About the optional inducible expression:

Lactamase was expressed under an optional tetracycline inducible suCMV promoter. All ready-to-use expression Lactamase particles can be used for constitutive high expression of Lactamase without using any induction. However, the particles can be optionally used as tetracycline induced expression when the tetracycline regulator protein (tetR) is present in advance. The tetR must be expressed in advance to stop the particles' transcription, and the expression is activated by addition of tetracycline (see the picture below for details). This inducible expression is tetracycline's dose dependent. In general, the amount of tetracycline is used at 1ug/ml final concentration. The image below illustrates how the inducible expression works.

If inducible expression is desirable, the repressor regulator (TetR) expression must be delivered in advance or at the same time for transduction. The presence of tetR can be achieved by the following methods:

- TetR is already expressed in a stable cell line that constantly express TetR protein in advance;
- Transfect a TetR expression plasmid before transduction of lactamase lentiviral particles;
- Co-transduce both the TetR repressor particles and the gene expression particles into the sample cells. The double transduced cells can be selected by double antibiotics, and then used for inducible expression. AMSBIO provides “**premade tetR particles**” with different antibiotics for double selection of transduced cells :

<http://www.amsbio.com/datasheets/LVP017-bsd.pdf>



Transduction Protocols:

1. Adhesive cells Transduction Protocols:

Day 0: Seed the desired cells in complete medium at appropriate density incubate overnight. (Note: at the time of transduction, it grows to 25% ~50% confluent.) For example, seed Hela cells at $0.5 \times 10^5/\text{ml} \times 0.5\text{ml}$ in a well of a 24-well plate;

Day 1: Thaw the Pre-made lentiviral stock at room temperature. Add appropriate amount of virus stock to obtain the desired MOI. Or simply add 50ul of virus into one well in 24-well plate without worry about the MOI number. Return cells to 37°C/CO₂ incubator. **A common used MOI number is 10.** (Note: Add polybrene to final concentration at 6-8 ug/ml may help the transduction to some cell lines.)

Day 3: At the time of ~72hr after transduction, Check the transduction rate via fluorescence image with a suitable filter under fluorescent Microscope, or calculate the exact transduction % rate via Flow Cytometry System (FACS) or any flow cytometry (such as Guava machine). (Note: Some cell lines need longer time, up to one week to see the transduction effects / the fluorescent signal.)

Day 3 + (optional): Transduced cell can be sorted out via FACS. Or you can select transduced stable cell line by a specific antibiotic (dependent upon the used particles types). A pilot experiment should be done to determine the antibiotic kill curve for your specific cell line.

2. Suspension cells transduction Protocols:

1. Grow your cell in your completed suspension culture medium, shaking in flask in CO₂ incubator;
2. Measure cell density. When cell grow to $\sim 3 \times 10^6$ cell/ml, measure cell viability (should > 90%), then diluted cells into 1×10^6 cell/ml in completed medium;
3. Transduction: thaw lentiviral particles at room temperature. Simply add premade lentiviral particle into the diluted cells at ratio of: **200ul virus per 2ml cells** (Note: depend upon the cell types; you may need to use more or less viruses). Grow cells in flask, shaking in CO₂ incubator.
4. At 24 hour after transduction, add equal amount of fresh medium containing final concentration of Blastidicin at 5 ~ 10ug/ml depend upon cell types (or other antibiotics dependent upon the particle types). Grow cell shaking in CO₂ incubator.
5. At 72 hours after transduction, check fluorescence under microscope or calculate the transduction efficiency using cell sorting machine (like FACS or Guava machine).

(Note: GFP filter wavelength: Ex450-490 ~Em525; RFP filter: ~Ex545/~Em620).

Safety Precaution:

Please use extra caution when using lentiviral particles. Remember. Wear glove all the time at handling Lentiviral particles! Please refer CDC and NIH's links (see references) for more details regarding to safety issues.

References:

1. Molecular Therapy (2003) 7, 460–466; doi: 10.1016/S1525-0016(03)00024-8
2. Annu Rev Microbiol. 1994;48:345-69.
3. Microbiol Mol Biol Rev. 2005 Jun;69(2):326-56.
4. NIH Guidelines for Biosafety Considerations for Research with Lentiviral Vectors.
5. CDC guidelines for Lab Biosafety levels (Link).

Warranty:

This product is warranted to meet its quality as described when used accordance with its instructions. AMSBIO disclaims any implied warranty of this product for particular application. In no event shall AMSBIO be liable for any incidental or consequential damages in connection with the products. AMSBIO's sole remedy for breach of this warranty should be, at AMSBIO's option, to replace the products.

These products are for research use only!

For general questions about our ready-to-use lentiviral particles, please consult our website: <http://www.amsbio.com/FAQ-Premade-Lentiviral-particles.pdf>

If you want to express or label your specific target, we also provide lentiviral **services for cloning your gene of interest** and generate ready-to-use viral particles with the best prices and fastest turnaround time. Please see our website for details: <http://www.amsbio.com/custom-lentivirus-service-expression-lentiviral.aspx>

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