GenTarget Inc

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Product manual: Pre-made Lentiviral Particles for GFP_lacZ fusion

Cat#	Product Name	Amounts
LVP021	GFP-lacZ (his) particles	200ul, ~1 x 10^7 IFU/mL in DMEM containing 10% FBS and 60ug/ml polybrene
LVP021-SF	Serum-free GFP-lacZ (his) particles	200ul, ~5 x 10^7 IFU/mL in serum-free medium

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

Product Description:

Lentiviral system is a gene delivery tool using lentivector for gene expression or knockdown. Lentivector is HIV-1 (Human Immunodeficiency Virus 1) derived plasmids. It produces lentiviral particles (lentivirus) that are capable to transduce into broad range of mammalian cell types or organs, including primary cells and non-dividing cells both in vivo and in cell culture system, and stably integrated into the transduced cell's genome, independent of cell cycle, for long term expression. Thus lentivirus holds unique promise as gene transfer agents.

Pre-made **GFP_LacZ fusion** lentiviral particles are generated from GenTarget's SureTiter lentiviral system. VSV-G pseudotyped lentiviral particles are generated in 293T cell. The particles are provided in two formats:

- packaged in 10% of FBS in DMEM containing 10% FBS and 60ug/ml of polybrene (Cat#:LVP021);
- packaged in serum-free medium without any human or animal origin components (Cat#: LVP021-SF);

The serum-free particles are best suitable for suspension cell transduction or serum sensitive cultures. For more details about premade particles, please see **FAQ for pre-made lentiviral particles** (.pdf).

Key features:

1. Lentiviral particles contain RFP-blasticidin resistant gene, allowing to generate GFP-lacZ fusion stable cell lines by Blasticidin antibiotic selection or via fluorescent cell sorting.
2. GFP fusioned with full length LacZ (β-Galactosidase) was expressed with a C-term His-tag.
3. The strongest suCMV promoter make the pre-made virus a ideal tool for mammalian protein expression, stable cell line construction and enzymatic assays both in vivo or in vitro (see schematic vector map below).

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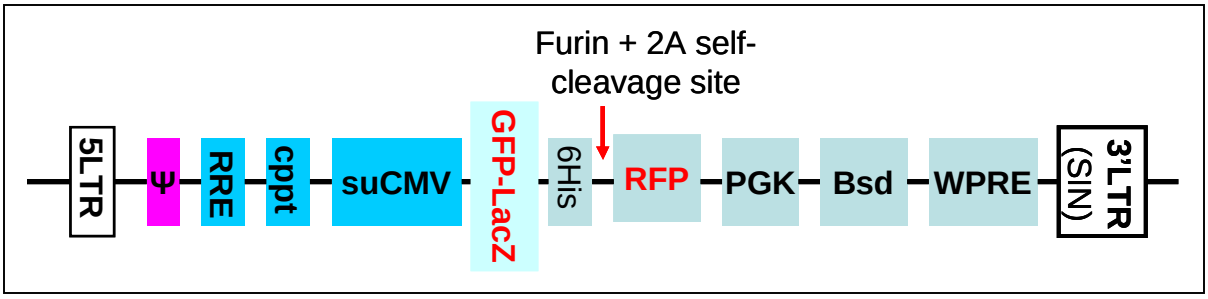
4. **The lentivirus are ready and easy to use, simply add 50ul into your cell culture.**



General Transduction Protocols:

1. Adhesive cells Transduction Protocols:

Day 0: Seed the cells in complete medium at appropriate density, incubate overnight.
(Note: at the time of transduction, it grows up to 10% ~50% confluent.)
For example, seed Hela cells at 0.5 x 10^5/ml x 0.5ml in a well of a 24-well plate;
Day 1: Remove the culture medium from the cells. Add fresh complete medium (Note: use as little media as possible at transduction). Thaw the Pre-made lentiviral stock at room temperature. Add appropriate amount of virus stock to obtain the desired MOI. Return cells to 37°C/CO2 incubator.
For example, add 50ul of lentiviral stock to the cells in 24-well plate above (getting MOI at 5).
Day 3: At ~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 Quava machine).
Day 3 + (optional): Transduced cell can be sorted out via FACS, or selected by Blastcidin antibiotic. A pilot experiment should be done to determine the kill curve for your specific cell line, Bsd ranged from 0.5ug ~10ug/ml.

2. Suspension cells transduction Protocols:

- Grow your cell in your completed suspension culture medium, shaking in flask in CO2 incubator;
- Measure cell density. When cell grow to ~3 x 10^6 cell/ml, measure cell viability (should > 90%), then diluted cells into 1 x 10^6 cell/ml in completed medium;
- Transduction: thaw lentiviral particles at room temperature. Simply add premade lentiviral particle into the diluted cells at ratio of: **100ul virus per 1ml cells** (Note: depend upon the cell types; you may need to use more or less viruses). Grow cells in flask, shaking in CO2 incubator.
- At 24 hour after transduction, add equal amount of fresh medium containing final concentration of Blasticidin at 5 ~ 10ug/ml depend upon cell types. Grow cell shaking in CO2 incubator. (Note: Gentarget's premade lentivirus contain Blasticidin

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resistance. So add Blasticidin antibiotic will enrich only the transduced cells for maximum protein production.)

- 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. 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.

References:

1. NIH stem cell training program (Link).
2. NIH Guidelines for Biosafety Considerations for Research with Lentiviral Vectors. (Link).
3. CDC guidelines for Lab Biosafety levels (Link).

Warranty:

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