



## Pre-made Lentiviral Particles for hP53 manual

**Product Name:** hP53 (His) Lentiviral particles  
**Cat#:** LVP020

**Amount:**

200ul,  $>5 \times 10^6$  IFU/mL in DMEM containing 10% FBS and 60ug/ml polybrene (10x);

**Storage:**  $<-70$  °C, avoid repeat freeze/thaw cycles.

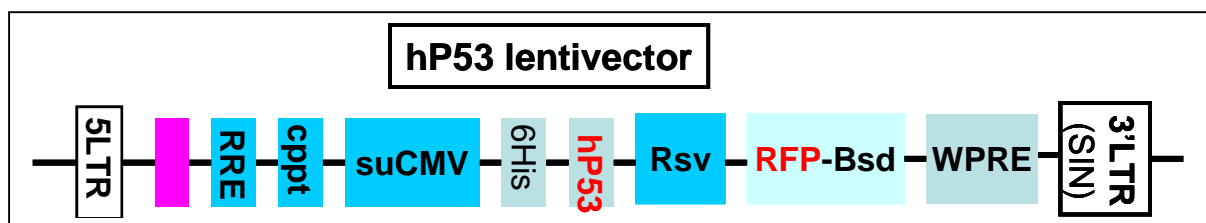
### Product Description:

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

Pre-made **hP53** lentiviral particles are generated from GenTarget's re-engineered lentivector system. Human **P53 gene** was fully verified by sequencing. VSV-G pseudotyped lentiviral particles are generated in 293T cell, and packed in 10% of FBS in DMEM, supplied as 200ul/per vial at  $> 5 \times 10^6$  IFU/ml containing 60ug/ml (10x) of polybrene. See [FAQ for pre-made lentiviral particles](#) (.pdf) for details.

### Key features:

1. Lentiviral particles contain RFP-blasticidin resistant gene, used for generating stable cell lines by Blasticidin antibiotics or via fluorescent cell sorting.
2. Target was expressed with a N-term His-tag for purification of target protein if desired.
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).
4. **The lentivirus are ready and easy to use, simply add 50ul into your cell culture.**





## 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 grow to 10% ~50% confluent.)

For example, seed Hela cells at  $0.5 \times 10^5$ /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/CO<sub>2</sub> incubator.

For example, add 5ul ~ 50ul of lentiviral stock to the cells in 24-well plate above (getting MOI from 0.5 to 5).

**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 Quava machine).

**Day 3 + (optional):** Transduced cell can be sorted out via FACS. Or you can select transduced stable cell line by Blastcidin. 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:

1. Grow your cell in your completed suspension culture medium, shaking in flask in CO<sub>2</sub> 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  $1 \times 10^6$  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 CO<sub>2</sub> incubator.
4. At 24 hour after transduction, add equal amount of fresh medium containing final concentration of Blastcidin at 5 ~ 10ug/ml depend upon cell types. Grow cell shaking in CO<sub>2</sub> incubator. (Note: Gentarget's premade lentivirus contain Blastcidin resistance. So add Blastcidin antibiotic will enrich only the transduced cells for maximum protein production.)
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. [NIH stem cell training program \(Link\)](#).
2. Takahashi, K. and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663-676.
3. Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A., Ruotti, V., Stewart, R., Slukvin, I.I., and Thomson, J.A. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318, 1917-1920.
4. Park, I.H., et al., Reprogramming of human somatic cells to pluripotency with defined factors. *Nature*, 2008. 451(7175): p. 141-6.
5. Shao, L., et al., Generation of iPS cells using defined factors linked via the self-cleaving 2A sequences in a single open reading frame. *Cell Res.*, 2009. 19(3): p. 296-306.
6. NIH Guidelines for [Biosafety Considerations for Research with Lentiviral Vectors](#). (Link).
7. [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.