



Premade Lentiviral Particles for iPS Stem Factors (mouse set)

For generating induced pluripotent stem (iPS) cells or other applications.

RESEARCH USE ONLY, not for diagnostics or therapeutics

Cat#	Product Name	amounts
LVP003m	m OCT4 (RFP-Bsd) inducible particles	200ul x (1x10 ⁸ IFU/ml)
LVP004m	m Sox2 (RFP-Bsd) inducible particles	200ul x (1x10 ⁸ IFU/ml)
LVP005m	m NANOG (RFP-Bsd) inducible particles	200ul x (1x10 ⁸ IFU/ml)
LVP006m	m LIN28 (RFP-Bsd) inducible particles	200ul x (1x10 ⁸ IFU/ml)
LVP007m	m Myc (RFP-Bsd) inducible particles	200ul x (1x10 ⁸ IFU/ml)
LVP008m	m Klf4 RFP-Bsd) inducible particles	200ul x (1x10 ⁸ IFU/ml)
LVP-stems- m-RB	Full set (6 mouse stem factors with RFP- Bsd marker)	200ul/ea x 6
LVP311m	m OCT4 (Neo) inducible particles	200ul x (1x10 ⁸ IFU/ml)
LVP312m	m Sox2 (Neo) inducible particles	200ul x (1x10 ⁸ IFU/ml)
LVP313m	m NANOG (Neo) inducible particles	200ul x (1x10 ⁸ IFU/ml)
LVP314m	m LIN28 (Neo) inducible particles	200ul x (1x10 ⁸ IFU/ml)
LVP315m	m Myc (Neo) inducible particles	200ul x (1x10 ⁸ IFU/ml)
LVP316m	m KLF4 (Neo) inducible particles	200ul x (1x10 ⁸ IFU/ml)
LVP-stems- m-N	Full set (6 mouse stem factors with Neomycin marker)	200ul/ea x 6

Storage: < -70 °C, avoid repeat freeze/thaw cycles. Products stable for 6 month.

Product Description:

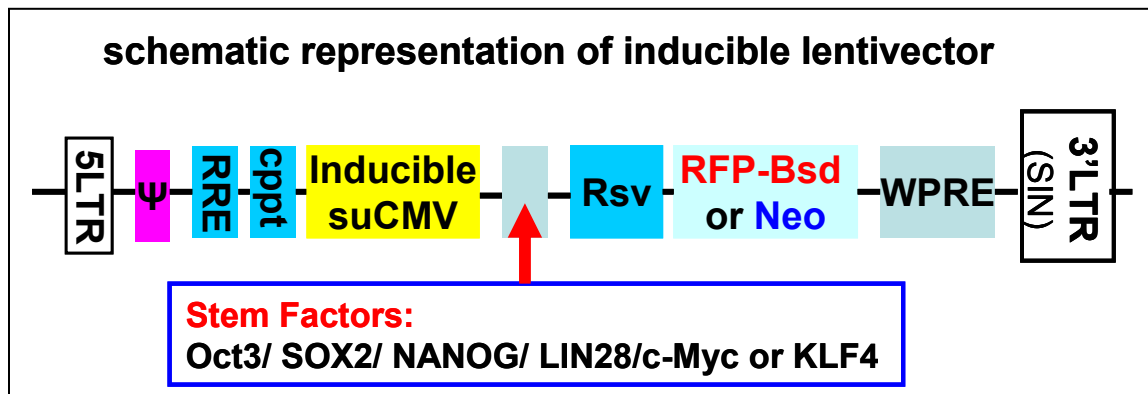
Lentiviral system is a gene delivery tool using lentivectors for gene expression or knockdown. Lentivectors are HIV-1 (Mouse Immunodeficiency Virus 1) derived



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.

Converting fully differentiated human or mouse somatic cells into embryonic-like cells (so called induced Pluripotent Stem Cell: iPSC) has attracted enormous attention in stem cell research. Multiple reports have demonstrated that iPSCs were generated by using a set of transcription factors or stem cell factors that delivered as expression virus or expressed proteins. Although the combination of reprogramming factors may slightly differ, the main stem cell factors are: OCT3/4, SOX2, NANOG, LIN28, cMyc and KLF4.

GenTarget pre-made lentiviral particles for iPSC are generated from its proprietary lentiviral vector system with either **RFP-Bsd** fusion dual marker or **Neomycin** marker (No RFP) (see vector map scheme below). Six **mouse** stem cell factors were first individually cloned into lentivector. Then, lentivectors were co-transfected with a packaging mix (Cat# **HT-pack**) into a 293T packaging cells (cat# **TLV-C**). The pre-made lentiviral particles are VSV-G pseudotyped virus, packaged in **serum-free** medium, and supplied as 200ul/per vial at $\sim 1 \times 10^8$ IFU/ml.

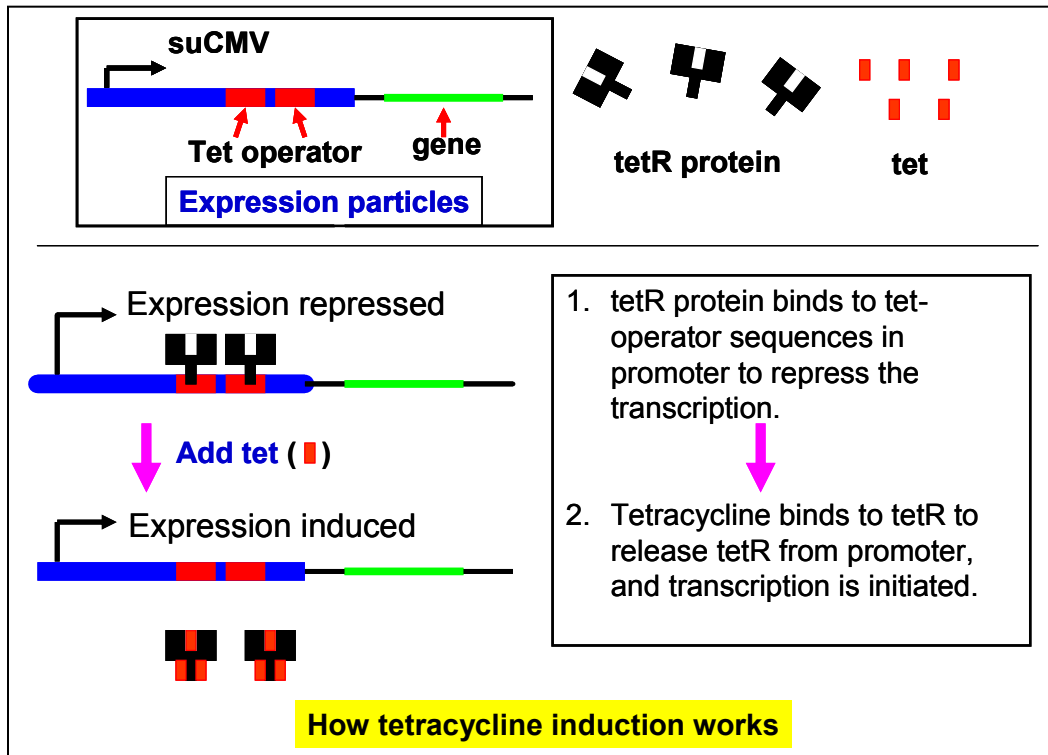


Each stem factor was natively expressed (without any tags) under a tetracycline inducible suCMV promoter in which two tetracycline operator sequences were integrated. **The particles can be used for regular constitutive high expression. And the same particles can also be used as tetracycline induced expression when the tetracycline regulator protein (tetR) is present in advance.** For inducible expression, the tetR must be expressed in advance to stop the transcription, and the expressed was activated by adding tetracycline. This inducible expression is tetracycline's dose dependent. In general, the amount of tetracycline is used at 1ug/ml final concentration. Please see the schematic instruction



(below) for the mechanism of inducible expression, and see our website for more details about [Inducible lentiviral system](#). Gentarget provide [premade lentivirus expressing TetR with different antibiotic markers](#). For particles general information, please refer to [FAQ about premade lentiviral particles](#). (<http://www.gentarget.com/pdf/FAQ-Premade-Lentiviral-particles.pdf>).

All six stem factor were sequencing verified. Their sequences fully match to the CD region according to the NCBI's database (see table below). Lentiviral particles contain blasticidin-RFP fusion marker, which allow to select the transduced cells by either fluorescence sorting or antibiotic selection.



Target	NCBI ID	Matched ORF position
m Myc	NM_010849.4	582--1946
m Klf4	NM_010637.3	605--2056
m Oct3/4	NM_013633.2	62--1120
m SOX2	NM_011443.3	412--1371
m LIN28	NM_145833.1	76--705
m NANOG	NM_028016.2	216--1133



Safety Precaution:

Please use extra caution when using lentiviral particles. Remember, you are dealing with transduction particles which can infection mouse cells. **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.

Related Product

Premade lentiviral particle for six human stem factors: Cat#:

[LVP003](#); [LVP004](#); [LVP005](#); [LVP006](#); [LVP007](#); [LVP008](#); [LVP-stems-h-RB](#);
[LVP311](#); [LVP312](#); [LVP313](#); [LVP314](#); [LVP315](#); [LVP316](#); [LVP-stems-h-N](#);
[LVP317 ~ LVP322](#); [LVP-stems-h-EF1a](#)

[LVP358](#): Chained human iPS (MKOS) inducible lentiviral particles

[LVP359](#): Chained mouse iPS (MKOS) inducible lentiviral particles

SC015	h Oct3/4 stable cells	2 x 10 ⁶ cells
SC016	h LIN28 stable cells	2 x 10 ⁶ cells
SC017	h NANOG stable cells	2 x 10 ⁶ cells

References:

1. [NIH stem cell training program \(Link\)](#).
2. Masaki Ieda, Ji-Dong Fu, et al. (2010). Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors. Cell 142, 375-386.
3. 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.
4. 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 mouse somatic cells. Science 318, 1917-1920.
5. Park, I.H., et al., Reprogramming of mouse somatic cells to pluripotency with defined factors. Nature, 2008. 451(7175): p. 141-6.
6. 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.
7. NIH Guidelines for [Biosafety Considerations for Research with Lentiviral Vectors](#). (Link).
8. [CDC guidelines for Lab Biosafety levels \(Link\)](#).

Attachment: iPS cells generation procedures:

Note: Human iPS cells were successfully generated from human patient fibroblast cells in 14 days using Gentarget Inc's human iPS lentivirus with this protocol. However, since each cell type is different, this protocol was for your reference only.

Day 0: Seed the parent cells:



For example, seed human fibroblast cells at 1×10^5 cells/well in 6-well plate, cultured in 5ml of growth medium, incubated overnight at 37°C with 5% CO₂.

Day 1: Viral Transduction:

On the second day, remove medium, add 1ml of pre-warmed fibroblast growth medium, then add 50 ul of each iPS lentivirus (Oct3/4, Sox2, NANOG, LIN28, c-Myc and Klf4, total 300ul of lentivirus) [Note: you may not need to use all iPS factors dependent upon your cell types. But reported showed this full set was successfully induced mouse adult fibroblast into iPSC cells]. Gentle mix for evenly distribution, then incubated overnight at 37°C with 5% CO₂.

Day 2: Optional: another transduction:

For some cell line, remove medium, and 1 ml of pre-warmed fibroblast growth medium, then add 50 ul of each iPS lentivirus for another transduction, incubated overnight at 37°C with 5% CO₂.

Day 3: Change Medium:

At about 48 hours after the 1st transduction, change medium with 5 ml fibroblast growth medium.

Day 5: Re-plate the transduced cells to feeder cells

At four Days of post-transduction, the cells were trypsinized, centrifuged at 200 x g for 5 minutes, resuspended in Fibroblasts Cell Growth Medium, and re-plated in a 150mm MEF Feeder Dishes. These cells were incubated overnight at 37°C with 5% CO₂.

Day 6: Change medium to reprogramming medium:

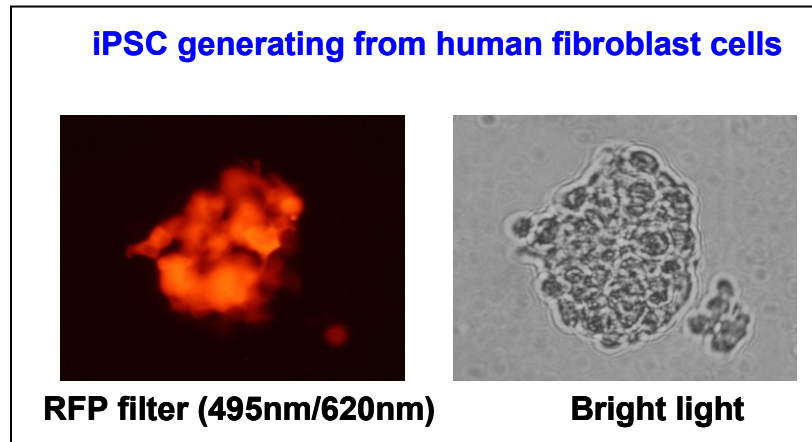
At 24 hours after re-seeding, Fibroblast Cell Growth Medium was replaced with Human ES/iPS Cell Medium (HiPS Cells Food). The medium was changed every day for the first 7 days. During this period, all ES cell-like colonies were selected and re-seeded in Human ES/iPS Cell Medium supplemented with 10 μM Stemolecule™ Y27632 on CF-1 MEF Plates. Human ES/iPS Cell Medium was changed every day. (Reprogramming Medium: HiPS food plus VPA, PD, SB and Tvz).

Day 13: Change to Condition Medium:



After re-programming in ES/iPS medium for 7 days, change medium into MEF Conditioned Medium. Continue to pass the cells until they showed typical human ES cell morphology.

iPS cell sample images:



iPS cells generated from human patient fibroblast cells by using GenTarget Inc's lentivirus set (Cat#: [LVP-stems-h](#))

