

Product Description

Product Name: LV-Fluc-P2A-Puro
 Catalog Number: LV012-S (0.25 mL) or LV012-L (1 mL)
 Lot Number: LV-IM2

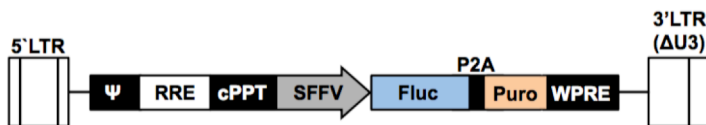
Reporter gene: Firefly luciferase (Fluc)
 Selection gene: Puromycin (Puro)

Quantity: 250 μ L
 Titer: 1.6×10^7 TU/mL*
 Storage media: Serum free media

Shipping: Dry ice
 Storage: Store at $\leq -70^\circ\text{C}$ upon receipt. Freeze-thaw cycles will decrease titer.

Shelf life: One year from date of receipt under proper storage conditions.

This is a ready-to-use lentivirus preparation. The virus encodes the firefly luciferase (Fluc) cDNA under control of the spleen focus-forming virus (SFFV) promoter linked to the puromycin resistance gene (Puro) via a P2A cleavage peptide (see below). The lentiviral vectors are self-inactivating (SIN) vectors in which the viral enhancer and promoter have been deleted. Transcription inactivation of the LTR in the SIN provirus increases biosafety by preventing mobilization by replication competent viruses and enables regulated expression of the genes from the internal promoters without *cis*-acting effects of the LTR¹.



5' LTR: 5' long terminal repeat
 ψ : RNA packaging signal
 RRE: Rev response element
 cPPT: Central polypurine tract
 SFFV: Spleen focus-forming virus promoter
 Fluc: Firefly luciferase
 P2A: P2A cleavage peptide
 Puro: Puromycin resistance gene
 WPRE: Woodchuck hepatitis virus posttranscriptional regulatory element
 3' LTR/ Δ U3: 3' self-inactivating long terminal repeat

*Titration by qPCR:

A WPRE probe-based qPCR assay was used to measure the number of copies of lentiviruses stably integrated into the genome after transduction of HeLa H1 cells (transducing units per mL).

Traditional p24 ELISA titrations measure both functional and non-functional lentivirus particles. However, this method overestimates the functional titer, as the p24 protein pool includes a variable amount of free p24 and p24 associated with non-functional vector particles. This ratio can vary greatly between each lot, so the titration is inherently inaccurate. While qPCR titers may appear lower than p24 ELISA, they are more accurate and functional.

Safety Precaution:

All culture work with lentiviruses should be performed by trained personnel and performed under BSL2 containment following NIH guidelines.



Basic Lentivirus Transduction Protocol

Volumes are given for a 6-well plate; increase or decrease as needed. See the Transduction Tips section for additional considerations/modifications.

1. Seed cells in complete medium at an appropriate density to achieve 60-70% confluency the next day (e.g. $\sim 2.5 \times 10^5$ HeLaH1 cells). Incubate cells overnight in a $37^\circ\text{C}/5\%$ CO_2 incubator.
2. Thaw lentivirus stock on ice.
3. In a microcentrifuge tube, dilute lentivirus to 1 mL total in serum free media. (See tips below for notes about determining optimal MOIs.)
4. Remove culture medium from cells and replace with prepared lentivirus.
5. Return cells to $37^\circ\text{C}/5\%$ CO_2 incubator.
6. After 4 hours add 1 mL complete medium to each well and return cells to $37^\circ\text{C}/5\%$ CO_2 incubator.
7. 3 days after transduction, check transgene expression according to an appropriate protocol. (Note: this lentivirus includes a selection gene; see tips below for details.)

Transduction Tips

1. To determine the optimal MOI for transductions: plate several wells of the target cells and infect with increasing MOIs (e.g. 1, 3, 10, and 30). Typically, primary cells require higher MOIs than established cell lines.
2. Polybrene[®] (Imanis #REA001) can be added to the transduction mixture to enhance transduction efficiency². The final concentration of Polybrene[®] in the transduction medium should be 4-8 $\mu\text{g}/\text{mL}$. Polybrene[®] can be cytotoxic to some cells and it is not advisable to incubate these cells with Polybrene[®] overnight; the transduction cocktail may be removed after 3-4 h and replaced with complete media.
3. A spin infection can also be used to increase transduction efficiency³. Once the transduction mixture is added to the cells, centrifuge the plate at 800 x g for 30 min at room temperature, before placing the cells in a $37^\circ\text{C}/5\%$ CO_2 incubator.
4. The presence of serum in the transduction mixture can greatly affect transduction efficiency⁴. In general, lower serum concentrations result in higher transduction efficiencies, though optimizing serum concentrations is recommended for each cell type.
5. The presence of the puromycin resistance gene facilitates selection of transduced cells with puromycin. Selection with puromycin can be performed before or after transgene testing. The appropriate concentration of puromycin to use for selection varies with each cell line and can be determined by performing a kill curve on parental in parallel with transduced cells.

References

- ¹Miyoshi et al. J Virol. 1998. 72:8150-8157.
- ²Konopka et al. J Gen Virol. 1991. 72: 2685-2696.
- ³O'Doherty et al. J Virol. 2000. 74:10074-10080.
- ⁴Andreadis and Palsson. Human Gene Ther. 1997. 8:285-291.

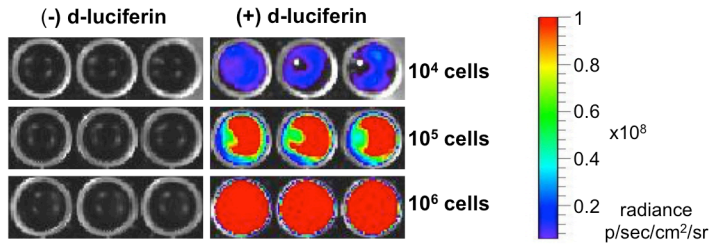
LV-Fluc-P2A-Puro

Certificate of Analysis

Testing performed by Imanis Life Sciences

Test description	Result
Virus titer	1.6×10^7 TU/mL
Sterility	No contamination detected
Puromycin selection	Pass QC
Luciferase expression	Pass QC

Luciferase Expression:



HeLaH1 cells were transduced with LV-Fluc-P2A-Puro (MOI = 10) and after three days the cells were amplified under puromycin selection. To measure luciferase expression, 10^4 , 10^5 , or 10^6 cells were placed in wells of a 96-well plate and 3 mg/mL of d-luciferin was added to the indicated wells. The plate was immediately imaged using the IVIS Spectrum imaging system.

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