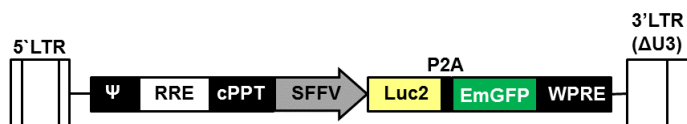


LV-Luc2-P2A-EmGFP

Product Description

| | |
|-----------------|--|
| Product Name: | LV-Luc2-P2A-EmGFP |
| Catalog Number: | LV050-S (0.25 mL) or LV050-L (1 mL) |
| Lot Number: | LV-IM65 |
| Reporter genes: | Firefly luciferase (Luc2) Emerald green fluorescent protein (EmGFP) |
| Promoter: | Spleen focus-forming virus (SFFV) |
| Quantity: | 1 mL (L) |
| Titer: | 6.68 x 10 ⁷ TU/mL* |
| Storage media: | Serum-free media |
| Shipping: | Dry ice |
| Storage: | Store at ≤ -70°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 (Luc2) cDNA linked to the emerald green fluorescent protein (EmGFP) cDNA via a P2A cleavage peptide; the spleen focus-forming virus (SFFV) promoter transcriptionally controls expression of the Luc2-P2A-EmGFP cDNA (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
 Luc2: Firefly luciferase
 P2A: P2A cleavage peptide
 EmGFP: Emerald green fluorescent protein
 WPRE: Woodchuck hepatitis virus posttranscriptional regulatory element
 3' LTR/ΔU3: 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. ~ 2.5 x 10⁵ HeLaH1 cells). Incubate cells overnight in a 37°C/5% CO₂ 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°C/5% CO₂ incubator.
6. After 4 hours add 1 mL complete medium to each well and return cells to 37°C/5% CO₂ incubator.
7. 3 days after transduction, check transgene expression according to an appropriate protocol.

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 μg/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°C/5%CO₂ 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.

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.

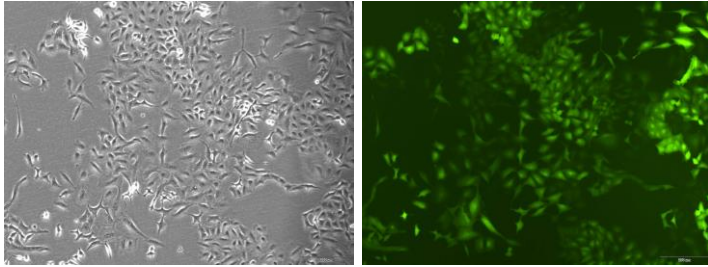
LV-Luc2-P2A-EmGFP

Certificate of Analysis

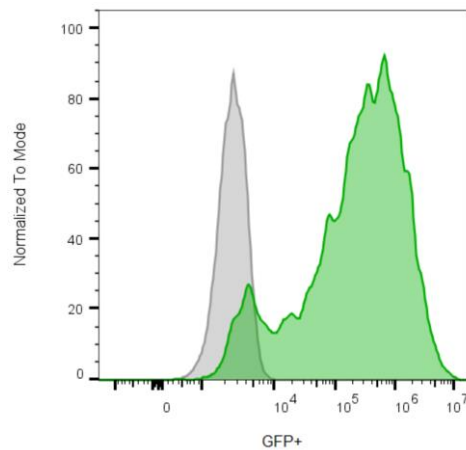
Testing performed by Imanis Life Sciences

| Test description | Result |
|-------------------------|------------------------------|
| Virus titer | 6.68 x 10 ⁷ TU/mL |
| Sterility | No contamination detected |
| Luciferase expression | Pass QC |
| Fluorescence expression | Pass QC |
| Endotoxin testing | Pass QC |

Fluorescence Expression:

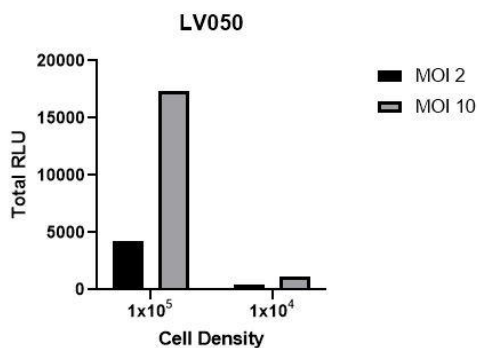


A549 cells were transduced with LV-Luc2-P2A-EmGFP (MOI=10). Cell photos were taken at 100x magnification 3 days after transduction.



A549 cells were transduced with LV-Luc2-P2A-EmGFP (MOI=10). The transduced cells (green) and untransduced control cells (grey) were fixed with paraformaldehyde and analyzed by flow cytometry.

Luciferase Expression:



A549 cells were transduced with LV-Luc2-P2A-EmGFP at the indicated MOIs. To measure luciferase expression, 10⁴ or 10⁵ cells were seeded in wells of a 96-well plate and 3 mg/mL of d-luciferin was added to the indicated wells. Luciferase activity was measured using a TECAN plate reader.

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