

## Product Description

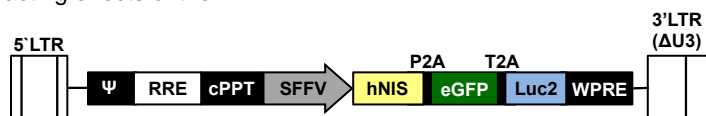
Product Name: LV-hNIS-P2A-eGFP-T2A-Luc2  
 Catalog Number: LV021-S (0.25 mL) or LV021-L (1 mL)  
 Lot Number: LV-IM27

Reporter genes: Human sodium iodide symporter (hNIS)  
 Enhanced green fluorescent protein (eGFP)  
 Firefly luciferase (Luc2)

Quantity: 250  $\mu$ L (S) or 1 mL (L)  
 Titer:  $6.49 \times 10^7$  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 human sodium iodide symporter (hNIS) cDNA linked to the enhanced green fluorescent protein (eGFP) cDNA via a P2A cleavage peptide and the firefly luciferase (Luc2) cDNA via a T2A cleavage peptide; the spleen focus-forming virus (SFFV) promoter transcriptionally controls expression of the hNIS-P2A-eGFP-T2A-Luc2 cDNA (see below). The lentiviral vector is a 2<sup>nd</sup> generation, self-inactivating (SIN) vector 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 LTR<sup>1</sup>.



5' LTR: 5' long terminal repeat  
 $\Psi$ : RNA packaging signal  
 RRE: Rev response element  
 cPPT: Central polypurine tract  
 SFFV: Spleen focus-forming virus promoter  
 hNIS: Human sodium iodide symporter  
 P2A: P2A cleavage peptide  
 eGFP: enhanced green fluorescent protein  
 T2A: T2A cleavage peptide  
 Luc2: Firefly luciferase  
 WPRE: Woodchuck hepatitis virus posttranscriptional regulatory element  
 3' LTR/ $\Delta$ 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.

## 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  $37^\circ\text{C}/5\% \text{CO}_2$  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  $37^\circ\text{C}/5\% \text{CO}_2$  incubator.
6. After 4 hours add 1 mL complete medium to each well and return cells to  $37^\circ\text{C}/5\% \text{CO}_2$  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**<sup>®</sup> (Imanis #REA001) can be added to the transduction mixture to enhance transduction efficiency<sup>2</sup>. The final concentration of Polybrene<sup>®</sup> in the transduction medium should be 4-8  $\mu\text{g}/\text{mL}$ . *Polybrene*<sup>®</sup> can be cytotoxic to some cells and it is not advisable to incubate these cells with *Polybrene*<sup>®</sup> 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 efficiency<sup>3</sup>. Once the transduction mixture is added to the cells, centrifuge the plate at  $800 \times g$  for 30 min at room temperature, before placing the cells in a  $37^\circ\text{C}/5\% \text{CO}_2$  incubator.
4. The presence of serum in the transduction mixture can greatly affect transduction efficiency<sup>4</sup>. In general, lower serum concentrations result in higher transduction efficiencies, though optimizing serum concentrations is recommended for each cell type.

## References

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

## Safety Precaution:

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



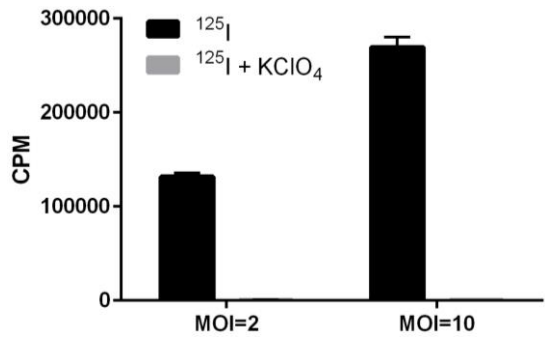
# LV- hNIS-P2A-eGFP-T2A-Luc2

## Certificate of Analysis

Testing performed by Imanis Life Sciences

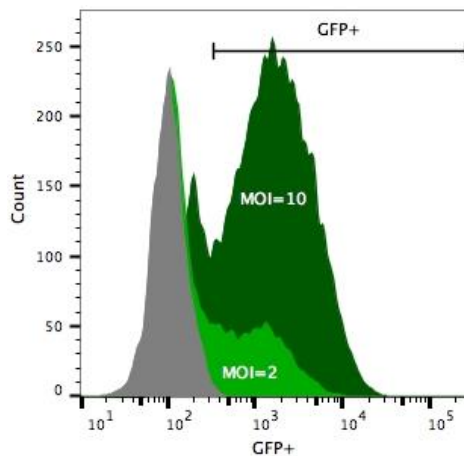
Test description	Result
Virus titer	6.49 x 10 <sup>7</sup> TU/mL
Sterility	No contamination detected
Endotoxin testing	Pass QC
NIS expression	Pass QC
Fluorescence expression	Pass QC
Luciferase expression	Pass QC

## NIS Expression:



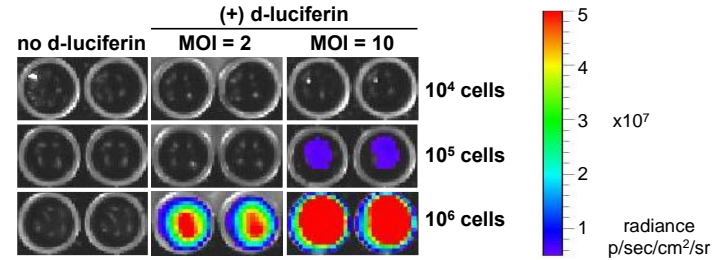
HeLaH1 cells were transduced with LV-hNIS-P2A-eGFP-T2A-Luc2 at the indicated MOIs. After 1 week <sup>125</sup>I uptake by the cells was measured in the presence or absence of KClO<sub>4</sub>, an inhibitor of NIS-mediated <sup>125</sup>I uptake.

## Fluorescence Expression



HeLaH1 cells were transduced with LV-hNIS-P2A-eGFP-T2A-Luc2 at the indicated MOIs. After 6 days fluorescence expression of the transduced cells (green) and untransduced parental HeLaH1 cells (grey) was analyzed by flow cytometry.

## Luciferase Expression:



HeLaH1 cells were transduced with LV-hNIS-P2A-eGFP-T2A-Luc2 at the indicated MOIs. To measure luciferase expression, 10<sup>4</sup>, 10<sup>5</sup>, or 10<sup>6</sup> cells were seeded 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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