LV-iRFP-P2A-Puro

Product Description

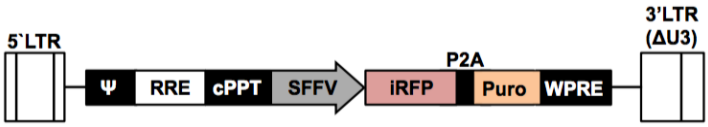
Product Name: LV-iRFP-P2A-Puro
Catalog Number: LV032
Lot Number: LV-IM54

Reporter gene: Near-infrared fluorescent protein (iRFP)
Selection gene: Puromycin (Puro)

Quantity: 1 mL
Titer: 2.7 x 10^7 TU/mL*
Storage media: Serum free media

Shipping: Dry ice
Storage: Store at ≤ -70ºC 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 near-infrared fluorescent protein (iRFP; ex/em = 690/713) cDNA linked to the puromycin resistance (Puro) cDNA via a P2A cleavage peptide; the spleen focus-forming virus (SFFV) promoter transcriptionally controls expression of the iRFP-P2A-Puro cDNA (see below). The lentiviral vector is a 2nd 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[1].



5' LTR: 5' long terminal repeat
ψ: RNA packaging signal
RRE: Rev response element
cPPT: Central polypurine tract
SFFV: Spleen focus-forming virus promoter
iRFP: Near-infrared fluorescent protein
P2A: P2A cleavage peptide
Puro: Puromycin resistance gene
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 10^5 HeLaH1 cells). Incubate cells overnight in a 37ºC/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 37ºC/5% CO_2 incubator.
6. After 4 hours add 1 mL complete medium to each well and return cells to 37ºC/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 efficiency[2]. 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 efficiency[3]. 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 37ºC/5%CO_2 incubator.
4. The presence of serum in the transduction mixture can greatly affect transduction efficiency[4]. 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

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

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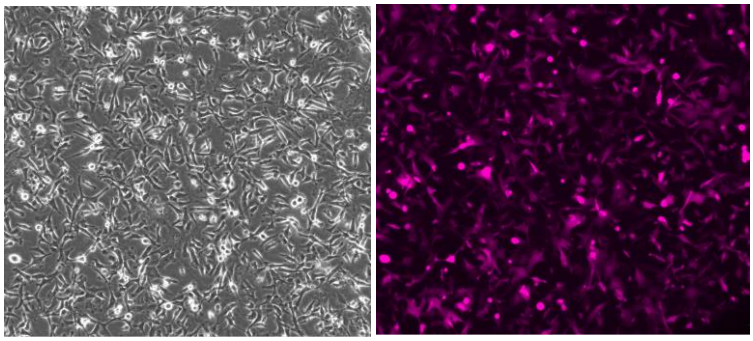
Certificate of Analysis

Testing performed by Imanis Life Sciences

Test description	Result
Virus titer	2.7 x 10^7 TU/mL
Sterility	No contamination detected
Endotoxin testing	Pass QC
Fluorescence expression	Pass QC
Puromycin resistance	Pass QC

Fluorescence Expression:



HT1080 cells were transduced with LV-iRFP-P2A-Puro (MOI = 10). Matched phase (*left*) and fluorescent (*right*) photos were taken at 100x magnification.

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