GenTarget Inc

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# Lentivirus for Nano-Lantern Luminescence Imaging

## (Bio-sensor / Bio-probe for *In Vivo* Imaging)

| Cat# | Product Name | Amounts |
|---|---|---|
| LVP1454 | Nano-Lantern **Nuclear Ca++** Bioprobe | 200ul x ($1x10^8$ IFU/ml)<br><br>in PBS solution, pre-mixed with 10x polybrene (60ug/ml). |
| LVP1522 | Nano-Lantern **Mitochondrial NAD+** Bioprobe | |
| LVP1523 | Nano-Lantern **Nuclear NAD+** Bioprobe | |
| LVP1524 | Nano-Lantern **Cytoplasmic NAD+** Bioprobe | |
| LVP1525 | Nano-Lantern **Nuclear Cas9** Probe | |
| LVP1526 | Nano-Lantern **Nuclear dCas9** Probe | |
| LVP1527 | Nano-Lantern **Zyxin** | |
| LVP1528 | Nano-Lantern **β-tubulin** | |
| LVP1529 | Nano-Lantern **hTERT** | |
| LVP1530 | Nano-Lantern **H2B** | |
| LVP1531 | Nano-Lantern **Nucleus** area | |
| LVP1532 | Nano-Lantern **Cytoplasm** area | |
| LVP1533 | Nano-Lantern Endoplasmic Reticulum (**ER**) | |
| LVP1534 | Nano-Lantern **Golgi** | |
| LVP1535 | Nano-Lantern **Mitochondria** | |
| LVP1536 | Nano-Lantern **Nuclear-Membrane** | |
| LVP1537 | Nano-Lantern **Microtubule** | |
| LVP1538 | Nano-Lantern **Lysosomes** | |
| LVP1539 | Nano-Lantern **Endosomes** | |
| LVP1540 | Nano-Lantern **plasma membrane** | |

**Storage:** **<** -70 °C, avoid repeat freeze/thaw cycles. Stable for > 6 months.

## Product Description

## 1. Introduction

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**Lentivirus** is an effective and easy method for delivering genes into most mammalian cell types, including non-dividing and primary cells. It allows for the integration of genes into the host cell genome for long-term expression.
**Nano-lantern Imaging** is a technology that achieves brighter luminescence signals through bioluminescence resonance energy transfer (**BRET**) from Renilla luciferase to a fluorescent protein. This technology was developed by Takeharu Nagai and colleagues at Osaka University.

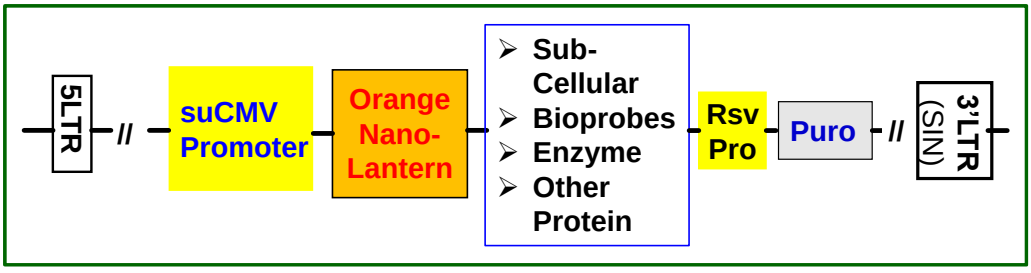
The brightness of Nano-lanterns is about 20 times greater than that of wild-type Renilla luciferase, although it is still over 100 times weaker than fluorescent proteins. However, Fluorescence Imaging requires external excitation laser light, which has drawbacks such as autofluorescence, phototoxicity, and photobleaching. Nano-Lantern does not require light activation and has an extremely low-level background signal, making it more sensitive and quantitative. Nano-lanterns are bright enough to be detectable as single molecules and can be continuously imaged for longer exposure times without photobleaching or photodamage. Additionally, when desired, Nano-Lantern can still be tracked as fluorescent proteins if excited with light activation (for example, use Ex 551nm for Orange Nano-Lantern).

## 2. Gentarget Inc's Nano-Lantern Bio-probes:

GenTarget has utilized its proprietary lentiviral vector systems to generate a set of **Orange Nano-Lantern Lentivirus** products. This Nano-Lantern consists of an enhanced Renilla-Luciferase connected to an Orange Fluorescent Protein (**OFP**). This Nano-Lantern structure then connect to a well-defined organelle sub-cellular targeting structure, or to a Bio-probe.

The specially designed Nano-Lanterns emit light in response to specific biological activities (such as **Ca++** dynamics, or **NAD+**) or for In Vivo luminescence imaging of sub-cellular structures.

The lentivirus also contains an the **Puromycin** selection marker under a Rsv promoter, which allow for selection of the lentivirus transduced cells when desired. (see lentivector's structure scheme below).



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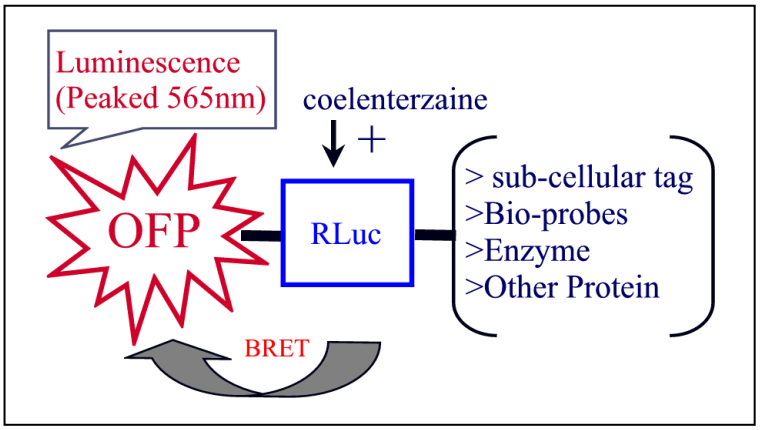
## 3. How Nano-Lantern Imaging works?

Nano-lanterns contain an enhanced Renilla luciferase fused to an Orange Fluorescent Protein (OFP). When supplied with the luciferase substrate, the luciferase transfers energy to the OFP, resulting in a fluorescent (luminescent) signal that peaks at 565nm. When these Nano-Lanterns are fused to a sub-cellular localization structure, a Bioprobe (such as Ca++ responsive element), or a target (such as Cas9 enzyme and so on), the localized luminescence can be detected or the dynamics of the target can be tracked. This makes it a great tool for real-time luminescent imaging of sub-cellular structural organization and dynamic processes in living cells and organisms. The following scheme represent its mechanism.




Since Nano-Lantern requires a molecular substrate instead of excitation light, its challenge is the supply and consumption of its substrate. Renilla-Luciferase uses coelenterazine as its substrate. A final concentration of 5 mM coelenterazine is commonly used for luminescent measurement. A modified, more sustained variant of this substrate can also be used.

Pre-made Nano-Lantern lentiviruses are extremely easy to use. Simply add the lentivirus to a mammalian cell culture (see procedure below). Positive transduced cells can be selected via Puromycin killing. Upon addition of coelenterazine substrate to the selected cells, the luminescent signal (peaking at 565nm) can be detected during a time-course in living cells.

For general questions about our ready-to-use lentiviral particles, please See *FAQs for pre-made lentiviral particles* (.pdf) on our website.

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## Transduction Protocols:

**Note:** Pre-made lentivirus is provided ready to use, so it can be simply added into your cell culture or inject into living animal. The amount of virus to add depends on cell type, or on your administration limit for animal assay.

For quick transduction, add 50 µl of virus into each well of 24-well-plate where cell density is 50% to 75%. After 48 hour to 72hours (no need to change medium), you can select the transduced cells by antibiotic killing, and then inject the cells into animal if desired.

**Day 0**:

Seed cells in complete medium at the appropriate density and incubate overnight.

**Note:** at the time of transduction, cells should be 50%-75% confluent. For example, seed HeLa cells at 0.5 x $10^5$/ml x 0.5ml in a well of a 24-well plate.

**Day 1**:

- Thaw the pre-made lentiviral stock at room temperature and add the appropriate amount of virus stock to obtain the desired MOI or just 50ul lentivirus/per well in 24w/p.
- Return cells to 37°C, $CO_2$ incubator.

**Note:** Try to avoid freezing and thawing for the lentivirus. If you do not use all of the virus at one time, you may re-freeze the virus at -80 °C for future use; virus titer will decrease by ~10% for each freeze/thaw cycle.

**Day 3+**:

At 48hr~72hr poat transduction (depend upon cell types), pass the cells and select the transduced cells if desired by puromycin killing. Then, the cells are ready for in vivo imaging upon the additions of coelenterazine substrate.

## Safety Precaution:

Gentarget lentiviral particles adapts must advanced lentiviral safety features (using the third generation vectors with self-inactivation SIN-3UTR), and the premade lentivirus is replication incompetent. However, please use extra caution when using lentiviral particles. Use the lentiviral particles in Bio-safety II cabinet. Wear glove all the time when handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

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## References:

1. *Nat Commun 2012;3:1262. doi: 10.1038/ncomms2248.*
2. *Proc Natl Acad Sci USA, 2015; 112(14):4352-4356.*
3. *Nat Chem Biol. 2019 May; 15(5): 433–436.*

## Warranty:

**This product is for research use only**. It is warranted to meet its quality as described when used in accordance with its instructions. GenTarget disclaims any implied warranty of this product for particular application. In no event shall GenTarget be liable for any incidental or consequential damages in connection with the products. GenTarget's sole remedy for breach of this warranty should be, at GenTarget's option, to replace the products.

**Attachment:** GenTarget's pre-made lentivirus product categories.

| Product Category | Product Description (please click into each category's page) |
|---|---|
| Pathway Reporter | Lentivirus for all kinds of pathway assays |
| Cell Immortalization | Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al. |
| ImmunoOncology Research | Lentivirus products for immuno therapy research, CAR-T, TCR-T, Assay cell lines, and Cell Antigens & Receptors. |
| CRISPR Gene Editing | Preamde lentivirus express humanzied wild-type **Cas9** endonuclease, the **dCas9**, gRNAs, **CRISPR** gene editing research |
| Cell-Specific Reporter | a set of reporter lentiviruses to express a luminescence or fluorescent reporter (firefly Luciferase, Renilla luciferase, RFP or GFP fluorescent marker) under a tissue specific promoter |
| Infectious Antigens | Llentivirus that express all kinds of infectious antigens with C-term 6His-tag. |
| Virus Like Particles (VLP) | Lentiviral Like Particles, pseudo-typed with a different envelope proteins. |
| Non-integrating LV | Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis. |

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| shRNA Knockdown | Knockdown verifeid and customized shRNA lentivirus for target knockdown, |
| microRNA lentivirus | Premade lentivirus expression human or mouse **precursor miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA. |
| Anti-miNA lentivirus | Pre-made lentivirus expression a specific anti-miRNA cassette. |
| Human and mouse ORFs | Premade lentivirus expressin a **human, mouse or rat** gene with RFP-Blastididin fusion dual markers. |
| Luciferase expression | Premade lentivirus for all kinds of luciferase protein expression**: firefly and Renilla, Red-Luc and more,** with different antibiotic selection markers. |
| Fluorescent Markers | Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, niRFP, unstable GFP and others. |
| Luminescent Imaging | Lentivirus express Nano-Latern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms |
| Cytoskeleton Imaging | A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure |
| Unstable GFP | Lentivirus express the the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway assay and in knockdown / knockout detection |
| near-infrared RFP | The near-infrared Red fluorescent (niRFP) expression Lentiviurs provides the whole-body images with better contrast and brighter images |
| Fluorescent-ORF fusion | Pre-made lentivirus expression a "**GFP/RFP/CFP-ORF**" fusion target. |
| CRE recombinase | Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers. |
| LoxP ColorSwitch | Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo. |
| SEAP Reporter | lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter), |
| TetR Repressor | Premade lentivirus expressin TetR (tetracycline |

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| | regulator) protein, the repressor protein for the inducible expression system. |
| rtTA Expression | rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF inducible system. |
| iPS factors | Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibitoic markers |
| LacZ expression | Express different full length **β- galactosidase (lacZ)** with different selection markers |
| Negative control lentiviruses | Premade **negative control lentivirus with different markers**: serves as the negative control of lentivurs treatment, for validation of the specificity of any lentivirus target expression effects. |
| Other Enzyme expression | Ready-to-use lentivirus, expressing a specific enzymes with different selection markers. |
| Ultra titer lentivirus | Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells. |
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