GenTarget Inc

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Llight inducible reporter lentiviruses

Cat#	Product Name	Amounts
Light activator lentivirus		
LVP473	Light activator (**puro**) Lentiviral particles	200ul, 1 x 10^8 IFU/mL, (in PBS (premixed with Polybrene)
LVP474	Light activator (**Bsd**) Lentiviral particles	
Light inducible reporter lentivirus		
LVP859-P	Light-inducible (C30)x5-**GFP** (Puro) Lentiviral particles	200ul, 1 x 10^7 IFU/mL in DMEM containing 10% FBS
LVP860-P	Light-inducible (C30)x5-**RFP** (Puro) Lentiviral particles	
LVP861-P	Light-inducible (C30)x5-**FLuc** (Puro) Lentiviral particles	
LVP862-P	Light-inducible (C30)x5-**Rluc** (Puro) Lentiviral particles	
LVP859-B	Light-inducible (C30)x5- **GFP** (Bsd) Lentiviral particles	
LVP860-B	Light-inducible (C30)x5- **RFP** (Bsd) Lentiviral particles	
LVP861-B	Light-inducible (C30)x5- **Luc** (Bsd) Lentiviral particles	
LVP862-B	Light-inducible (C30)x5- **Rluc** (Bsd) Lentiviral particles	
LVP859-N	Light-inducible (C30)x5- **GFP** (Neo) Lentiviral particles	
LVP860-N	Light-inducible (C30)x5- **RFP** (Neo) Lentiviral particles	
LVP861-N	Light-inducible (C30)x5- **Luc** (Neo) Lentiviral particles	
LVP862-N	Light-inducible (C30)x5- **Rluc** (Neo) Lentiviral particles	
LVP859-R	Light-inducible (C30)x5- **GFP** (**RFP**) Lentiviral particles	
LVP861-R	Light-inducible (C30)x5- **Luc** (**RFP**) Lentiviral particles	
LVP862-R	Light-inducible (C30)x5- **Rluc** (**RFP**) Lentiviral particles	
LVP860-G	Light-inducible (C30)x5- **RFP** (GFP) Lentiviral particles	
LVP861-G	Light-inducible (C30)x5- **Luc** (GFP) Lentiviral particles	
LVP862-G	Light-inducible (C30)x5- **Rluc** (GFP) Lentiviral particles	

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

Product Description:

Lentiviral system is a gene delivery tool using lentivectors for gene expression or knockdown. GenTarget's lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced into almost all kinds of mammalian cells, including stem cells, primary cells, and non-dividing cells both *in vivo* and *in vitro*. Lentiviral Particles stably integrate into the transduced cells' genome for long term expression, making it a great gene transfer agent.

This **light-dependent report system** contains a light inducible promoter and a light activator protein that trigger the inducible promoter's expression. The illumination triggers the photochemical formation changes of light-activator that consists of two domains: LSD (light-sense doman) and DBD (DNA binding domain), allowing its dimerization which binds to light inducible promoter, and turns on the downstream report expression. When turn back to dark, this formation change reverse back, rapidly inactivate report expression.

GenTarget developed a set of reporting lentivirus for **Blue light** (465 nm) dependent expression. Those reporting lentivirus has a luminescent report or a fluorescent report under the Light-inducible promoter (that contains **5x C30 tandem repeats** as the transcriptional response element). This promoter is not active until a **light-activator** binding to it.

The **light-activator** is photosensitive protein derived from Erythrobacter litoralis protein EL222[1]. It consists of two domains, the light sensor domain (**LSD** or called **LOV**: light-oxygen-voltage) and the DNA binding domain (*DBD* or called **HTH**: helix-turn-helix). In the dark, the two domain are interacted with each other to prevent its binding to "light inducible promoter sequence". When blue light is on (465nm), the illumination breaks these interactions (protein formation changes), opening the DBD domain from LSD domain[2], which triggers the DBD binding to the light-inducible promoter sequence, and turn on the downstream report expression (see mechanism schematic figure below).

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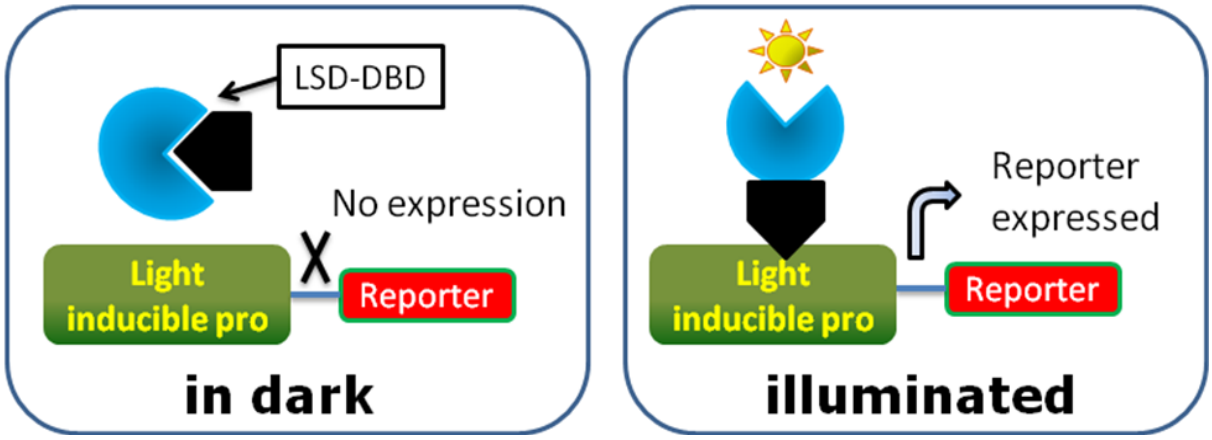
Light-switch reporter system:



- **Principle**:

  Cells are double transduced with light-activator lentivirus and light-inducible lentivirus, then, upon the Blue light irradiation, cells will express the luminescent or fluorescent report, which can be easily readout via luciferase assay or by fluorescent microscope. The light-activator lentivirus contains either Puromycin or Blasticidin antibiotic selection marker. The light-inducible reporting lentivirus contains a fluorescent selection marker or an antibiotic selection marker under a constitutive RSV promoter. The different antibiotic resistance allow the selection of the stably infected light-inducible signal reporting cell lines.

  Those reporting lentivirus products provide the efficient and easy tools to research light-inducible expression mechanism in vitro and in vivo.

- **Product Features**:

1) Rapid and nontoxic inducible expression specifically by Blue light illumination, and rapid deactivation in the dark;
2) Reversible (induction / deactivation) expression;
3) Precisely timed and localized induction;

Premade Blue light inducible lentivirus:

For the light-activator lentivirus: GenTarget provides two version products with different antibiotic selection marker, puromycin (CAT#: **LVP473**) and Blasticidin (CAT#: **LVP474**). Those activators are carefully engineered with high efficient nuclear penetration leader, NLS (nuclear

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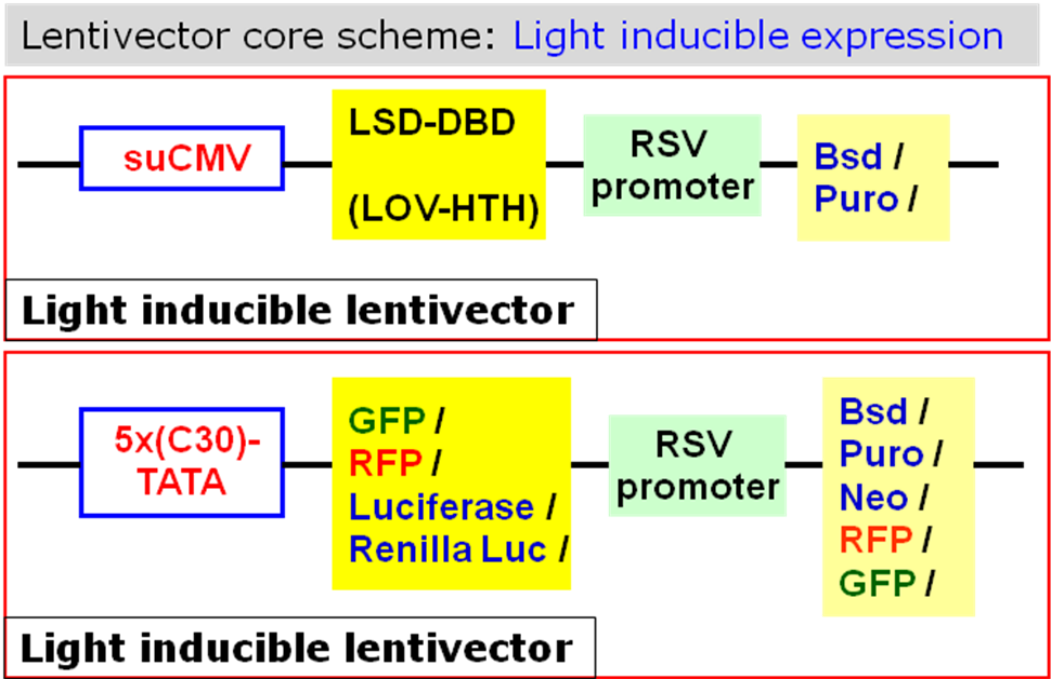
localization sequence) for maximum activation inside nuclear once illuminated. It specifically activated by blue light only.

For the Light-inducible-reporter lentivirus: GenTarget provides four different reporter system, **GFP, RFP, firefly Luciferase** and **Renilla luciferase** with different antibiotic selection marker (Blasticidin, puromycin or Neomycin, or a different fluorescent marker from the reporter). Each report is driven under the light-inducible promoter that contains the five repeats of light-activator protein's binding sequence [(C30)x5]. The promoter is not active until the light-activator protein binds to it. And the light-activator protein can bind to it only when there is blue-light illumination, which resulted in the downstream reporter's expression [1][3]. See the schematic map below for the core structure of the light-inducible reporter lentivectors.



Lentivirus was pseudotyped with VSVG envelope protein, produced in 293T cells. All particles were tested to be free bacterial and mycoplasma contamination. Virus titers were tested lot by lot.

The lentivirus are ready and easy to use, simply add 50ul into one well of your cell culture in 24-well plate, and select or sort the positive transduced cells at 2-3 days post virus transduction. The readout can be easily monitored

by luciferase assay or via the Fluorescent microscope or Fluorescent-readers depending on product report type.

Ready-to-use luciferase lentiviral particles are provided in two formats:

1. Packaged in 10% of FBS in DMEM containing 10% FBS and 60ug/ml of polybrene (10x);
2. Particles were concentrated and buffer exchanged in PBS without any human or animal origin components. The virus in PBS is good for any cell types that requires non-serum in the medium, or good for hard-to-infect cell types.

For more details about premade particles, please see FAQ for pre-made lentiviral particles (.pdf).

Protocol notes:

1) Pre-made lentivirus is provided ready to use, so it can be simply added into your cell culture; the amount of virus to add depends on cell type. For quick transduction, add 50 µl of virus into each well of 24-well-plate where cell density is 50% to 75%. After 72 hours (no need to change medium), visualize positive transduction rate by fluorescence microscopy. For stable cell line generation, pass cells into medium containing antibiotic or perform fluorescence cell sorting followed by antibiotic selection.

2) The light induced expression peaks at about 12 hour illumination cycle time, elevated a few folds to a few hundred folds above the non-illuminated controls, and stay at high expression plateau as long as the light stimulation keeps on. When light turns off, the expression will be deactivation from 30 seconds up to a few hours. The exact activation and deactivation kinetics has to be tested in your specific assay cell types.

3) Procedure outline for light inducible expression:
   1. Select one Light-activator lentivirus with desired antibioic marker, and one light-inducible lentivirus with a different marker.
   2. Infect cells with both light-activator lentivirus and light-inducible lentivirus. For transient expression, proceed to next step; For stable expression cell line, select the double transduced cells;
   3. Illuminate the cells by light pulse irradiation: use 8 ~10 W /m^2 blue light (465 nm) with 20 seconds on and 60 seconds off cycles for 12 to 24 hour;
   4. Measure / Observe the elevated reporter's expression by luciferase assay (for Luc reporters) or via Fluorescent microscope (for fluorescent

reporters), compared to the non-illuminated cells to quantify the induction rate / folds.

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. Ware glove all the time at handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

References:

1. Biochemistry. 2012 December 18; 51(50): 10024–10034.
2. P.N.A.S, 2011 June 7, V(108):9449-9454;
3. Nature Chemical Biology, 2014, January 12. 10.1038
4. News of The Week, 2015 October 19, V93 (41): 8;

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.

Note: Filter wavelength settings:

BFP filter: ~Ex380 ~Em460; CFP filter: ~Ex436 ~Em480;
GFP filter: ~Ex450-490 ~Em525; YFP filter: ~Ex500 ~Em535;
RFP filter: ~Ex545 ~Em620;

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

Product Category	Product Description (please click into each category's page)
Pathway Reporter	Repoter Lentivirus for all kinds of pathway screening 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 and TCR; Assay Cell Lines for T-cell targeted killing assay and other cell-based assays; over-expression lentivirus products for the immune response targets; Cell surface antigens (CDs); immune checkpoint /

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Product Category	Product Description (please click into each category's page)
	Receptors; CRISPR gene Repair and knock-IN lentivirus; CRISPR knockout lentivirus;
CAR-T, TCR Lentivirus	**CARs** Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; **TCRs**: MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRαV3-F2A-TRβV5-6;
CRISPR Gene Editing	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease, the **dCas9**, gRNAs, **CRISPR** gene editing research
Epigenomic: CRISPRi and CRISPRa	"**dCas9-Protein**" fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).
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.
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

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Product Category	Product Description (please click into each category's page)
	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
Sub-cellular Imaging	Lentivirus contain a well-defined organelle targeting signal fusioned to a fluorescent protein, great tools for live-cell imaging and for dynamic investigation of sub-cellular signal pathways.
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.
CRE, Flp ColorSwtich	Lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" or "FRT-GFP-Stop-FRT-RFP" cassette, used to monitor the CRE or Flp 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 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

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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.