GenTarget Inc

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Lentivirus for Epigenomic Expression Regulation via CRISPR

Cat#	Product Name	Amounts
LVP1546	dCas9-**KRAB** (**Puro**) Lentivirus	200ul/vial, [1 x 10^8 IFU/mL, in PBS solution, premixed with 10x (60ug/ml) of polybrene]
LVP1547	dCas9-**KRAB** (**Bsd**) Lentivirus	
LVP1548	dCas9-**KRAB** (**Neo**) Lentivirus	
LVP1549	dCas9-**KRAB** Lentivirus	
LVP1550	dCas9-DNMT3a (**Puro**) Lentivirus	
LVP1551	dCas9-DNMT3a (**Bsd**) Lentivirus	
LVP1552	dCas9-DNMT3a (**Neo**) Lentivirus	
LVP1553	dCas9-DNMT3a Lentivirus	
LVP1554	dCas9-**DNMT3b** (**Puro**) Lentivirus	
LVP1555	dCas9-**DNMT3b** (**Bsd**) Lentivirus	
LVP1556	dCas9-**DNMT3b** (**Neo**) Lentivirus	
LVP1557	dCas9-**DNMT3b** Lentivirus	
LVP1558	dCas9-DNMT1 (**Puro**) Lentivirus	
LVP1559	dCas9-DNMT1 (**Bsd**) Lentivirus	
LVP1560	dCas9-DNMT1 (**Neo**) Lentivirus	
LVP1561	dCas9-DNMT1 Lentivirus	
LVP1562	dCase-**APOBEC** (**Puro**) Lentivirus	
LVP1563	dCase-**APOBEC** (**Bsd**) Lentivirus	
LVP1564	dCase-**APOBEC** (**Neo**) Lentivirus	
LVP1565	dCase-**APOBEC** Lentivirus	
LVP1566	dCas9-RT (**Puro**) Lentivirus	
LVP1567	dCas9-RT (**Bsd**) Lentivirus	
LVP1568	dCas9-RT (**Neo**) Lentivirus	
LVP1569	dCas9-RT Lentivirus	
LVP1570	dCas9-VP64-SunTag (**Puro**) Lentivirus	
LVP1571	dCas9-VP64-SunTag (**Bsd**) Lentivirus	
LVP1572	dCas9-VP64-SunTag (**Neo**) Lentivirus	
LVP1573	dCas9-VP64-SunTag Lentivirus	
LVP1574	dCas9-**P65** (**Puro**) Lentivirus	
LVP1575	dCas9-**P65** (**Bsd**) Lentivirus	
LVP1576	dCas9-**P65** (**Neo**) Lentivirus	
LVP1577	dCas9-**P65** Lentivirus	
LVP1578	dCas9-HSF1 (**Puro**) Lentivirus	
LVP1579	dCas9-HSF1 (**Bsd**) Lentivirus	
LVP1580	dCas9-HSF1 (**Neo**) Lentivirus	
LVP1581	dCas9-HSF1 Lentivirus	

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LVP1582	dCas9-**GAL4** (**Puro**) Lentivirus	
LVP1583	dCas9-**GAL4** (**Bsd**) Lentivirus	
LVP1584	dCas9-**GAL4** (**Neo**) Lentivirus	
LVP1585	dCas9-**GAL4** Lentivirus	
LVP1586	dCas9-GFP (**Puro**) Lentivirus	
LVP1587	dCas9-GFP (**Bsd**) Lentivirus	
LVP1588	dCas9-GFP (**Neo**) Lentivirus	
LVP1589	dCas9-GFP Lentivirus	
LVP1590	dCas9-RFP (**Puro**) Lentivirus	
LVP1591	dCas9-RFP (**Bsd**) Lentivirus	
LVP1592	dCas9-RFP (**Neo**) Lentivirus	
LVP1593	dCas9-RFP Lentivirus	
LVP1594	dCas9-Null (**Puro**) Lentivirus	
LVP1595	dCas9-Null (**Bsd**) Lentivirus	
LVP1596	dCas9-Null (**Neo**) Lentivirus	
LVP1597	dCas9-Null Lentivirus	

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

Product Description:

1. **About Gentarget Inc's Lentiviral System**:

   GenTarget's Lentivector system is based on Human Immunodeficiency Virus-1 (HIV) lentivector plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced into most mammalian cell types, 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 lentivirus an excellent gene transfer agent.

2. **About CRISPR and dCas9**:
   Cas9 from Streptococcus pyogenes is the most frequently used Cas endonuclease in CRISPR. dCas9 (deficient Cas9 or Dead Cas9) is a mutant of spCas9. It lacks the ability to cleave double-stranded DNA but is still capable of cleaving only one strand of the genome DNA (so-called "nickase"). Like Cas9, dCas9 can still be localized to the desired genome site guided by specific RNAs (gRNA), thus binding to the targeted genomic DNA sites.

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GenTarget constructed dCas9 by making two mutations (D10A and H840A) in spCas9. The D10A mutation is located in the RuvC domain of the Cas9 protein, which is responsible for cleaving the non-target DNA strand. This mutation impairs the endonuclease activity of Cas9 by disrupting the coordination of the Mg2+ ion required for catalysis. The H840A mutation is located in the HNH domain, which is responsible for cleaving the target DNA strand. This mutation greatly reduces its cleavage activity. Therefore, Cas9 can still cleave only one strand (the PAM site strand) of the DNA double helix but with reduced efficiency compared to wild-type Cas9 nuclease. This dCas9 was constructed with a nuclear localization sequence (**NLS**) and in framed **HA tag** so the expression of dCas9 can be detected by anti-HA tag if desired.

3. **Gentarget's CRISPRi and CRISPRa Lentivrus products**:

The dCas9 protein binds to a specific genomic location under the guidance of a target site-specific gRNA. When coupled with a protein, the resulted "dCas9-Domain" fusion can modify the genome sequence by preventing the cell's transcription machinery from accessing the binding region, activating promoters, or inducing CRISPR interference (CRISPRi) or activation (CRISPRa). The activation domains recruited to the specific target site by the dCas9 protein and gRNA, do not affect the expression of other genes in the genome. This makes it an excellent tool for epigenomics analysis.

For instance, a dCas9 fused with a transcriptional activation domain can stimulate gene expression without modifying the DNA sequence. Alternatively, a dCas9 fused with a transcriptional repressor domain, such as KRAB, can suppress gene expression. In addition, dCas9 can modify local chromatin structures and make the genomic target sites accessible to other enzymes (such as methyltransferases or nucleases) for epigenetic regulation of gene expression. The "dCas9-transcription domain" system is also useful for screening assays that identify genes leading to a desired phenotype upon activation or repression. Refer to the illustration scheme below for CRISPR-based gene regulation.

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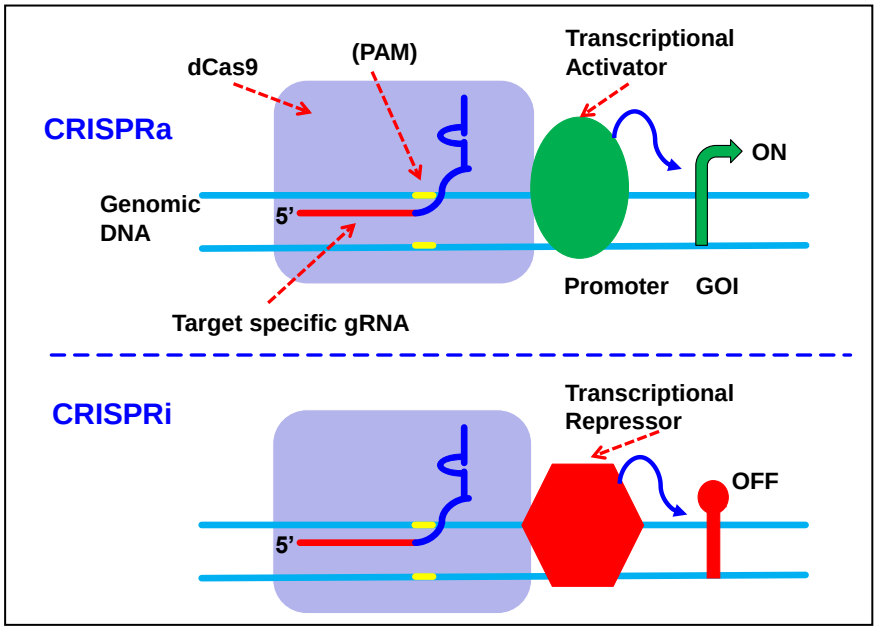


1) Interference via CRISPR (**CRISPRi**)

- **KRAB** (Kruppel-associated box domain):
  KRAB is protein domain is known for its ability to repress the activity of nearby genes by recruiting chromatin-modifying enzymes to the DNA, acting as a transcriptional repressor. The dCas9-KRAB system is a powerful tool for transcriptional repression of genes within their endogenous genomic loci.

- **Methyltransferase**:
  In mammals, DNMT1, DNMT3A and DNMT3B, are the generally recognized three types of DNA methyltransferases (DNMTs). The dCas9-DNMTs system has potential applications in epigenomic methylation of the targeted genome sequence such as promoter region, being a powerful gene editing tool for gene repression.

2) Activation via CRISPR (**CRPSPRa**)

- **VP64-SunTag**:
  The VP64 domain is derived from the herpes simplex virus VP16 protein and contains four copies of a 64-amino-acid sequence that can recruit various transcriptional co-activators to enhance gene transcription. The SunTag is multiple copies of SGSG peptide tag, separated by a flexible linker (GGGGG). SunTag allows for multiple

copies of an activation domain to be recruited to the target site for efficient gene activation. The "dCas9-SunTag-VP64" fusion creates a highly potent transcriptional activator for the targeted genes.

- **P65**:
  The human p65 (RELA) protein is a subunit of the transcription factor NF-κB. The "dCas9-p65" fusion can be used in regulation of NF-kB pathway. Under the guidance of gRNA, dCas9 transport the p65 to the targeted locus where to activate the transcription of specific target genes in a controlled manner.

- **HSF1**:
  The human heat shock factor 1 (HSF1) is a transcription factor. The "dCas9-HSF1" fusion combines the DNA-targeting capabilities of dCas9 with the transcriptional activation function of HSF1. It can bind to the heat shock response elements (HSEs) in the promoter regions of genes, resulting in the transcriptional upregulation of the targeted genes. The activity of HSF1 can be regulated by external stimuli such as heat shock or chemical inducers. By using the dCas9-HSF1 fusion, researchers can achieve inducible and reversible control of gene expression.

- **GAL4**:
  The GAL4 activation domain is derived from the yeast transcription factor GAL4 (galactose-responsive transcription factor). The "dCas9-GAL4" fusion recruit various co-activators, including the p300/CBP histone acetyltransferase, to enhance gene transcription.

3) Repair via **Reverse-transcriptase**
dCas9 only nick the genome DNA, generating the nick site for reverse-transcriptase based genome repair. The "dCas9-Reverse Transcriptase" fusion was constructed to coupe dCas9 with M-MLV Reverse Transcriptase Domain (with RNase H minus and three desired mutations for the enhanced Reverse Transcriptase's fidelity and activity). It can be a powerful genomic sequence repair tool.

4) Base change via **APOBEC**
The APOBEC1 (apolipoprotein B mRNA editing enzyme catalytic subunit 1) enzyme can then convert a cytidine base (C) to a uridine base (U) on the non-targeted strand of the DNA helix, creating a mismatched base pair. The target-specific gRNA can guide the "dCas9-APOBEC" fusion to the

targeted locus. The DNA repair machinery of the cell recognizes the mismatched base pair and replaces the U with T on the non-targeted strand and the A with a G base on the targeted strand, resulting in an A to G base change. The "dCas9-APOBEC" fusion system provides a powerful genomic editing tool for single base changes.

5) Trace dCa9 localization in genomic locus via **fluorescent** marker
The dCa9 coupled with a GFP or RFP fluorescent marker, provides a in vivo fluorescent-imaging tool for visualizing specific genomic loci in real-time in living cells. (Note: you need confocal fluorescent microscope for such visualization).

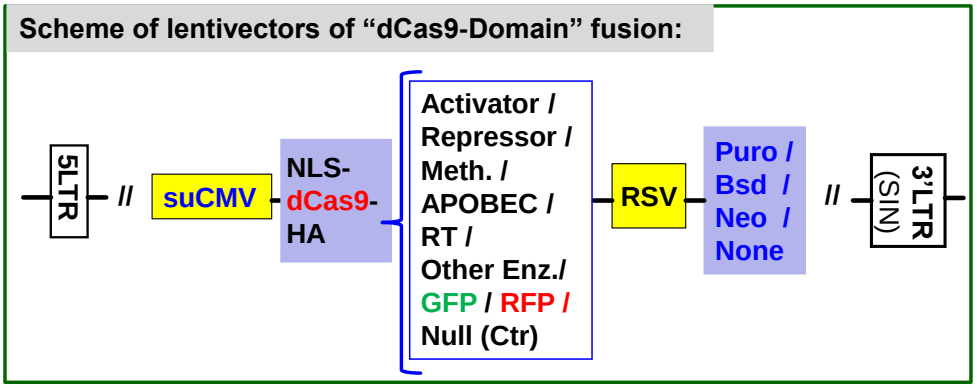
Note: Filter wavelength settings:
GFP filter: ~Ex450-490 ~Em525;
RFP filter: ~Ex558 ~Em583;

Product Features:

1) "dCas9-Domain" Lentivector structure:

The "dCas9-Domain" fusion lentivectors are constructed using the proprietary super CMV promoter (suCMV) that has highest promoter strength in most cell types, with a nuclear-localized sequence (NLS) and HA tag. The expression of dCas9 can be detected using anti-HA antibodies if desired. The lentivectors contain an antibiotic selection marker (Puromycin, Blasticidin, or Neomycin) under the Rsv promoter or do not include the antibiotic selection marker (No antibiotic). The following scheme represents the core lentivector structure.

Scheme of lentivectors of "dCas9-Domain" fusion:



The control lentivectors has the dCas9 coupling with a Null-sequence. Each control lentivirus has the same lentivector backbone as the dCas9-Domain

fusion has, with the coresponding antibiotic selection or no selection. Please see "*FAQs about premade lentiviral particles*" for more information on premade lentivirus in general.

2) Premade CRISPRa and CRISPRi lentivirus:

Gentarget offers a comprehensive collection of CRISPRi and CRISPRa Lentiviurs products, all premade, ready to use, provided in PBS solution, premixed with 60ug/ml of polybrene (10x), for in vitro and in vivo applications.

- Expression repression via KRAB;
- Promoter methylation via different Methyltransferase;
- Genomic base change via APOBEC;
- Genomic base repair via Reverse-transcriptase;
- Genomic frame shift via DNA Polymerase;
- Expression activation via VP64-SunTag, p65, HSF1, GAL4
- and more Epigenomic modification via other Domains;

The lentivirus adapted the most advanced biosafety features, including the self-inactivation feature in its 3' LTR, and only generates the replication-incompetent lentivirus.

3) Key Features:

- Driven by an extremely strong suCMV promoter, the lentivirus allows for high target expression levels.
- Using the gene-specific gRNA lentivirus (not included and must be designed and produced separately), the lentivirus can specifically target any gene.
- The lentivirus offers flexible selection through the use of a desired antibiotic, making it ideal for generating stable cell lines for genomic-wide screening assays.
- The lentivirus is ready-to-use and easy-to-use. Simply add it to your cell culture without any other reagents required. It can transduce most mammalian cell types, including primary cells, and can also be used directly for in vivo applications.

Transduction Protocols:

Note: 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.

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.
- Return cells to 37°C, CO_2 incubator. Do nothing.

Note: Try to avoid freezing and thawing. 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 (Depend upon cell type) post transduction, pass the cells for selection if desired, via antibiotic killing.

Day 3 +

Carry out the targeted CRISPRi or CRISPRa assays by applying gene specific gRNA lentivirus (Note: you need to design your own gRNA for your desired targets, and produce the gRNA lentivirus. If you like, Gentarget Inc and make any customized gRNA lentivirus for your GOI throught service orders. Please Email us with your request if you like).

Safety Precaution:

The Gentarget lentiviral particles are designed with advanced lentiviral safety features, utilizing third-generation vectors with self-inactivation (SIN-3UTR). Additionally, the premade lentivirus is replication-incompetent. However, it is crucial to exercise extra caution when handling lentiviral particles. It is recommended to use the lentiviral particles in a biosafety level II cabinet and wear gloves at all times when handling them. For more details on safety issues, please refer to the guidelines provided by the CDC and NIH.

References:

1. dCas9-KRAB: Nature Methods volume 12, pages1143–1149 (2015)
2. Nature Reviews Molecular Cell Biology volume 17, pages 5–15 (2016)
3. Methylation: Nucleic Acids Research, Volume 45, Issue 17, 29 September 2017, Pages 9901–9916,
4. Stem Cell Reports j Vol. 5 j 448–459 j September 8, 2015
5. Human Gene Therapy (2003) 14: 1089-105.
6. NIH Guidelines for Biosafety Considerations for Research with Lentiviral Vectors. (Link).

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 Products:

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 / 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

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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 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.

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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
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	Ultra-titer lentivirus used for the hard-to-transduced

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Product Category	Product Description (please click into each category's page)
lentivirus	cells and for in vivo manipulation of sperm cells, or stem cells.