GenTarget Inc

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Integration Defective Lentivirus (IDLV)

Cat#	Product Name	Amounts
IDLV001	**GFP** (CMV, Puro) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV002	**GFP** (EF1a, Puro) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV003	**GFP** (CMV, Bsd) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV004	**GFP** (EF1a, Bsd) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV005	**GFP** (CMV, **Neo**) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV006	**GFP** (EF1a, Neo) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV007	**Cas9** (CMV, Puro) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV008	**Cas9** (EF1a, Puro) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV009	**Cas9** (CMV, Bsd) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV010	**Cas9** (EF1a, Bsd) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV011	**Cas9** (CMV, Neo) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV012	**Cas9** (EF1a, Neo) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV013	**Cas9** (CMV, **GFP**-Puro) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV014	**Cas9** (EF1a, **GFP**-Puro) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV015	**Cas9** (CMV, **RFP**-Puro) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV016	**Cas9** (EF1a, **RFP**-Puro) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV017	**Cas9** (CMV, **GFP**-Bsd) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV018	**Cas9** (EF1a, **GFP**-Bsd) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV019	**Cas9** (CMV, **RFP**-Bsd) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV020	**Cas9** (EF1a, **RFP**-Bsd) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)
IDLV021	**Cas9** (CMV, Zeo) Integrase-Deficient Lentivirus	200ul, (1×10^7 IFU/mL)

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IDLV022	**Cas9** (EF1a, Zeo) Integrase-Deficient Lentivirus	200ul, (1 x 10^7 IFU/mL)
IDLV023	**Cas9** (CMV, no selection) Integrase-Deficient Lentivirus	200ul, (1 x 10^7 IFU/mL)
IDLV024	**Cas9** (EF1a, no selection) Integrase-Deficient Lentivirus	200ul, (1 x 10^7 IFU/mL)
IDLV025	**CRE** (CMV, Puro) Integrase-Deficient Lentivirus	200ul, (1 x 10^7 IFU/mL)
IDLV026	**CRE** (EF1a, Puro) Integrase-Deficient Lentivirus	200ul, (1 x 10^7 IFU/mL)

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

Product Description:

1. Introduction

GenTarget's lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene expression, knockdown and CRISPR gene editing. Compared to other virus-type gene delivery method, lentivirus showed the highest transduction rate in most mammalian cell types, and able to transduce primary cells, dividing and non-dividing cells, both *in vivo* and *in vitro*.

Pseudotyped lentivirus became the most effective research tool used to deliver gene products. However, for many applications, the genomic integration of Lentivirus brings in the risk of insertional mutagenesis. Therefore, the Integration Defective Lentivirus (IDLV) became an ideal solution, special in clinical and therapeutic applications.

2. Gentarget's IDLV products:

Pseudotyped lentivirus are produced by co-transfection the transfer plasmid (containing the gene products for delivery) with the packaging plasmids (which normally containing multiple plasmids). Lentivirus genomic integration is dependent upon the HIV-1 integrase which is part of the polyprotein precursor gag-pol. The HIV-1 integrase has been shown to interact with the cellular factor lensepithelium derived growth factor (LEDGF), which tethers the reverse-transcribed viral DNA to active genes thereby leading to their integration near such loci.

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To produce Integration Defective (or Deficient) lentivirus, different mutations were made in HIV-1 integrase (in one of the packaging plasmids). The best mutation was tested as the point mutation at D64V [Ref. 1], which reduces 97% integration with limited reduction in the lentivirus transduction rate. Gentarget constructed the IDLV packaging system containing D64V mutation in integrase. Using this special packaging system, Gentarget produces VSVG pseudotyped IDLV product lines, to satisfy the need for Non-integration lentivirus. Gentarget's IDLV products were verified with >95% integration reduction, via IDLV-GFP virus in 4-week cell expansion course

Gentarget IDLV products maintain the most advanced, third-generation Bio-safety features, such as self-inactivation (SIN) and more. All IDLV products are replication-deficient lentivirus.

3. Why use Integration Defective Lentivirus (IDLV)?:

1) IDLV avoid the risks of Insertional Mutagenesis, but retains lentivirus' high transduction efficiency in most mammalian cell types, and able to transduce both dividing and non-diving cells.

2) Like regular lentivirus, IDLV is best for transient over-expression, knockdown, CRISPR gene therapy in primary cells, or in "hard-to-delivery" cell types via other methods (for example, the lipid transfection or electroporation). Dislike regular lentivirus, IDLV does not have the unwanted footprint, the integrated stably expression.

3) Lentivirus can activate host's immune response against the transuded stably expressed foreigner protein. However, the IDLV, as transient expression, effectively reduces the host's immune rejection response to lentivirus application.

4) Compared to other non-integrating virus delivery method (like AAV or Adenovirus), IDLV is capable deliver large gene and has the highest transduction rate for most hard-to-transduced cell types (has the best cell tropism).

4. Gentarget Inc's IDLV Product Features:

The following scheme showed the transfer lentivector's core structure:

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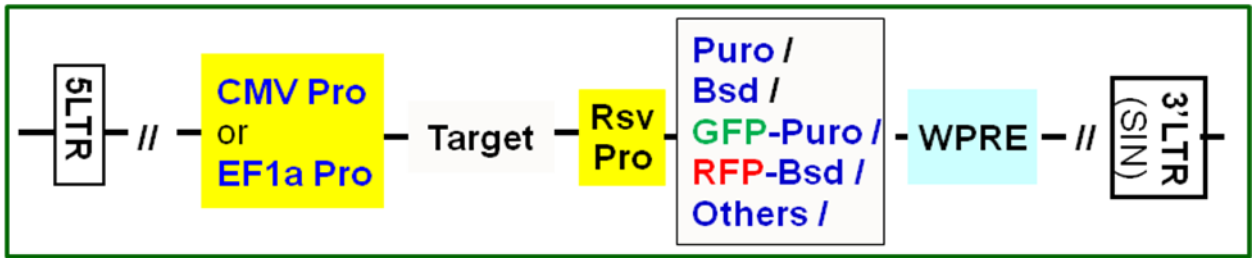

1) **Promoter option**: Transgene was driven by different promoters to best fit your cell types. The enhance **CMV** promoter has the highest promoter strength in most cell types; The modified **EF1a** promoter strength is much less cell type dependent, and perform better for the cell types where CMV promoter is weak.
2) **Transient selection:** Gentarget's IDLV carries an antibiotic marker or a Fluorescent-Antibiotic dual marker which provides a transient selection of the transduced cells, via antibiotic killing or via Fluorescent cell sorting. Once transduced, the IDLV becomes episomal circular form and diluted through cell divisions until it is diminished. The transduced cells still can be selected, if desired, within the 2 to 4 cell expansion. (The selection ability good for 2 to 3 weeks dependent cell dividing speed, and for many months or years for non-dividing cells).
3) **Product Format**: Gentarget's IDLV lentivirus are provided in as **200ul** of concentrated lentivirus in PBS with titer at ~1 x10^7 IFU/ml.

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

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5. Gentarget Inc's IDLV sample results:

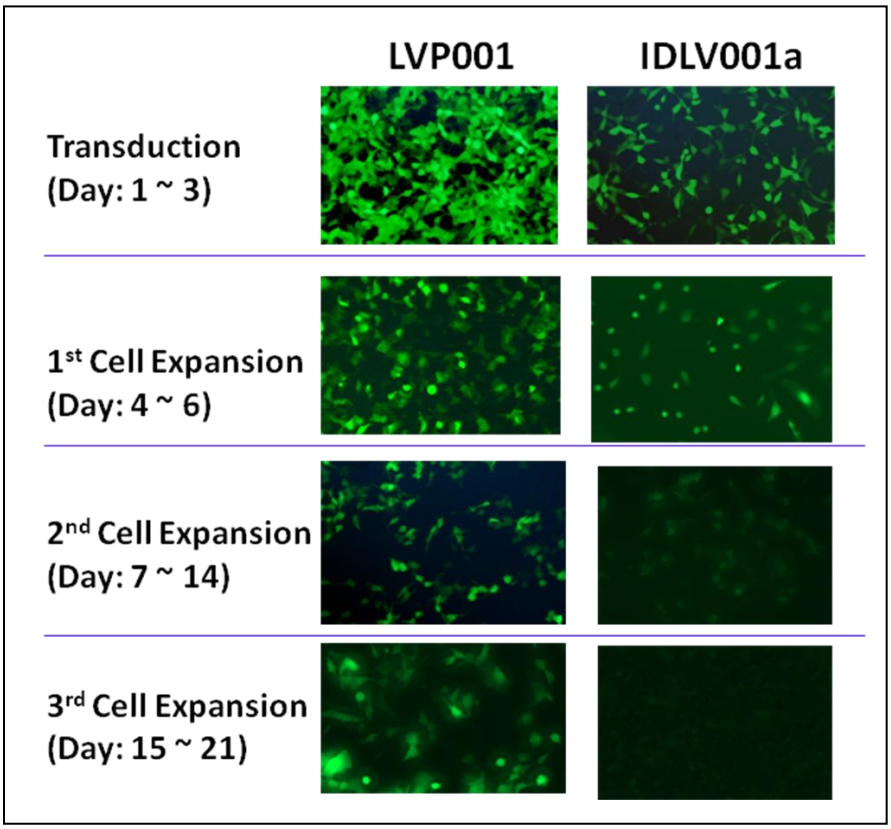

Figure 1: Compare regular GFP Lentivirus (CAT#: LVP340, Left Panel) and Integration Defective GFP Lentivirus (CAT#: IDLV001A, Right Panel). Both GFP Lentivirus was separately transduced into HEK293 cells. Images were taken during cell expansion. The episomal expressed IDLV GFP showed progressive GFP signal lost during cell dividing. The regular integrating GFP does not show GFP signal lost.

Transduction Protocols:

1. Transduction Protocol for Adhesive cells:

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.

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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 ^oC for future use; virus titer will decrease by ~10% for each freeze/thaw cycle.

Day 3:

At 48hr~72hr (Depend upon cell type) after transduction, check the transduction rate by fluorescence microscopy or calculate the exact transduction rate by flow cytometry (FACS or Guava).

Day 3 + (optional):

Sort transduced cells by FACS, or select by antibiotic killing. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line (refer to the pertinent literature on generation of stable cell lines).

2. Transduction Protocol for Suspension Cells:

Grow cells in complete suspension culture medium; use a shaking flask in a CO^2 incubator if required.

Measure cell density (not grow over 3 million/ml), measured viability should be > 90%. Dilute cells into 1 x 10^6 cell/ml in complete medium.

Day 1:

- Thaw lentiviral particles at room temperature.
- Add premade lentiviral particles into the diluted cells at a ratio of: 50 to 100 µl virus per 0.5 ml of cells (Note: depending on cell type, you may need to use more or less virus).
- Grow cells in a shaking flask in a CO2 incubator.

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Day 2:

At 24 hours after transduction, add an equal amount of fresh medium containing. Continue growing cells in CO2 incubator.

Day 3+:

At 48 hour to 72 hours (Depend upon cell type) after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Pass cells into 0.5 million/ml density in completed medium containing the corresponding antibiotic (**Note:** amount of antibiotic depends on cell type. A killing curve must pre-established). Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

Note: Filter wavelength settings:

GFP filter: ~Ex450-490; ~Em525; RFP filter: ~Ex545; ~Em590~610nm;

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.

References:

1) Naldini, L., et al. (1996). Science 272: 263 – 267.
2) Philippe, S., et al (2006) Proc. Natl. Acad. Sci. USA 103,17684–17689.;
3) Nightingale et al (2006) Mol. Ther 13(6):1121-1132;
4) Chamsy Sarkis et al (2008) Current Gene Therapy, 2008, 8, 430-437

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.

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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 / 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,

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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.
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.

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Product Category	Product Description (please click into each category's page)
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 lentivirus	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.