



## Pre-made Expression Lentivirus for Secreted alkaline phosphatase (SEAP)

Cat#	Product Name	Amounts
<a href="#">LVP1184</a>	SEAP (TetCMV, Puro) expression lentivirus	200 ul x (1x10 <sup>7</sup> IFU/ml)
<a href="#">LVP1185</a>	SEAP (TetCMV, Bsd) expression lentivirus	
<a href="#">LVP1186</a>	SEAP (TetCMV, Neo) expression lentivirus	
<a href="#">LVP1187</a>	SEAP (TetCMV, RFP-Bsd) expression lentivirus	
<a href="#">LVP1188</a>	SEAP (TetCMV, RFP-Puro) expression lentivirus	
<a href="#">LVP1189</a>	SEAP (TetCMV, GFP-Bsd) expression lentivirus	
<a href="#">LVP1190</a>	SEAP (TetCMV, GFP-Puro) expression lentivirus	
<a href="#">LVP1191</a>	SEAP (TetCMV, Hygro) expression lentivirus	
<a href="#">LVP1192</a>	SEAP (TetCMV, Zeo) expression lentivirus	
<a href="#">LVP1215</a>	SEAP (TetCMV, GFP) expression lentivirus	
<a href="#">LVP1216</a>	SEAP (TetCMV, RFP) expression lentivirus	
<a href="#">LVP1193</a>	SEAP (EF1a, Puro) expression lentivirus	
<a href="#">LVP1194</a>	SEAP (EF1a, Bsd) expression lentivirus	
<a href="#">LVP1195</a>	SEAP (EF1a, Neo) expression lentivirus	
<a href="#">LVP1196</a>	SEAP (EF1a, RFP-Bsd) expression lentivirus	
<a href="#">LVP1197</a>	SEAP (EF1a, RFP-Puro) expression lentivirus	
<a href="#">LVP1198</a>	SEAP (EF1a, GFP-Bsd) expression lentivirus	
<a href="#">LVP1199</a>	SEAP (EF1a, GFP-Puro) expression lentivirus	
<a href="#">LVP1200</a>	SEAP (EF1a, Hygro) expression lentivirus	



<a href="#">LVP1201</a>	SEAP (EF1a, Zeo) expression lentivirus	
<a href="#">LVP1217</a>	SEAP (EF1a, GFP) expression lentivirus	
<a href="#">LVP1218</a>	SEAP (EF1a, RFP) expression lentivirus	
<a href="#">LVP1202</a>	SEAP (CAG, Puro) expression lentivirus	
<a href="#">LVP1203</a>	SEAP (CAG, Bsd) expression lentivirus	
<a href="#">LVP1204</a>	SEAP (CAG, Neo) expression lentivirus	
<a href="#">LVP1205</a>	SEAP (CAG, RFP-Bsd) expression lentivirus	
<a href="#">LVP1206</a>	SEAP (CAG, RFP-Puro) expression lentivirus	
<a href="#">LVP1207</a>	SEAP (CAG, GFP-Bsd) expression lentivirus	
<a href="#">LVP1208</a>	SEAP (CAG, GFP-Puro) expression lentivirus	
<a href="#">LVP1219</a>	SEAP (Ubc, Puro)	
<a href="#">LVP1220</a>	SEAP (mPGK, Puro)	
<a href="#">LVP1221</a>	SEAP (ActB, Puro)	
<a href="#">LVP1184-PBS</a>	SEAP (TetCMV, Puro) lentivirus in PBS	200 ul x (1x10 <sup>8</sup> IFU/ml)
<a href="#">LVP1185-PBS</a>	SEAP (TetCMV, Bsd) lentivirus in PBS	
<a href="#">LVP1186-PBS</a>	SEAP (TetCMV, Neo) lentivirus in PBS	
<a href="#">LVP1187-PBS</a>	SEAP (TetCMV, RFP-Bsd) lentivirus in PBS	
<a href="#">LVP1188-PBS</a>	SEAP (TetCMV, RFP-Puro) lentivirus in PBS	
<a href="#">LVP1189-PBS</a>	SEAP (TetCMV, GFP-Bsd) lentivirus in PBS	
<a href="#">LVP1190-PBS</a>	SEAP (TetCMV, GFP-Puro) lentivirus in PBS	
<a href="#">LVP1191-PBS</a>	SEAP (TetCMV, Hygro) lentivirus in PBS	
<a href="#">LVP1192-PBS</a>	SEAP (TetCMV, Zeo) lentivirus in PBS	



<a href="#">LVP1215-PBS</a>	SEAP (TetCMV, GFP) lentivirus in PBS
<a href="#">LVP1216-PBS</a>	SEAP (TetCMV, RFP) lentivirus in PBS
<a href="#">LVP1193-PBS</a>	SEAP (EF1a, Puro) lentivirus in PBS
<a href="#">LVP1194-PBS</a>	SEAP (EF1a, Bsd) lentivirus in PBS
<a href="#">LVP1195-PBS</a>	SEAP (EF1a, Neo) lentivirus in PBS
<a href="#">LVP1196-PBS</a>	SEAP (EF1a, RFP-Bsd) lentivirus in PBS
<a href="#">LVP1197-PBS</a>	SEAP (EF1a, RFP-Puro) lentivirus in PBS
<a href="#">LVP1198-PBS</a>	SEAP (EF1a, GFP-Bsd) lentivirus in PBS
<a href="#">LVP1199-PBS</a>	SEAP (EF1a, GFP-Puro) lentivirus in PBS
<a href="#">LVP1200-PBS</a>	SEAP (EF1a, Hygro) lentivirus in PBS
<a href="#">LVP1201-PBS</a>	SEAP (EF1a, Zeo) lentivirus in PBS
<a href="#">LVP1217-PBS</a>	SEAP (EF1a, GFP) lentivirus in PBS
<a href="#">LVP1218-PBS</a>	SEAP (EF1a, RFP) lentivirus in PBS
<a href="#">LVP1202-PBS</a>	SEAP (CAG, Puro) lentivirus in PBS
<a href="#">LVP1203-PBS</a>	SEAP (CAG, Bsd) lentivirus in PBS
<a href="#">LVP1204-PBS</a>	SEAP (CAG, Neo) lentivirus in PBS
<a href="#">LVP1205-PBS</a>	SEAP (CAG, RFP-Bsd) lentivirus in PBS
<a href="#">LVP1206-PBS</a>	SEAP (CAG, RFP-Puro) lentivirus in PBS
<a href="#">LVP1207-PBS</a>	SEAP (CAG, GFP-Bsd) lentivirus in PBS
<a href="#">LVP1208-PBS</a>	SEAP (CAG, GFP-Puro) lentivirus in PBS
<a href="#">LVP1219-PBS</a>	SEAP (Ubc, Puro) lentivirus in PBS



<a href="#">LVP1220-PBS</a>	SEAP ( <b>mPGK</b> , <b>Puro</b> ) lentivirus in PBS	
<a href="#">LVP1221-PBS</a>	SEAP ( <b>ActB</b> , <b>Puro</b> ) lentivirus in PBS	

**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.

SEAP (Secreted Embryonic Alkaline Phosphatase) is a truncated form of human placental alkaline phosphatase (PLAP) through the deletion of a GPI anchor. It is secreted into cell culture supernatant and therefore offers many advantages over intracellular reporters, like luciferase. It allows to determine reporter activity without disturbing the cells, does not require the preparation of cell lysates and can be used for kinetic studies.

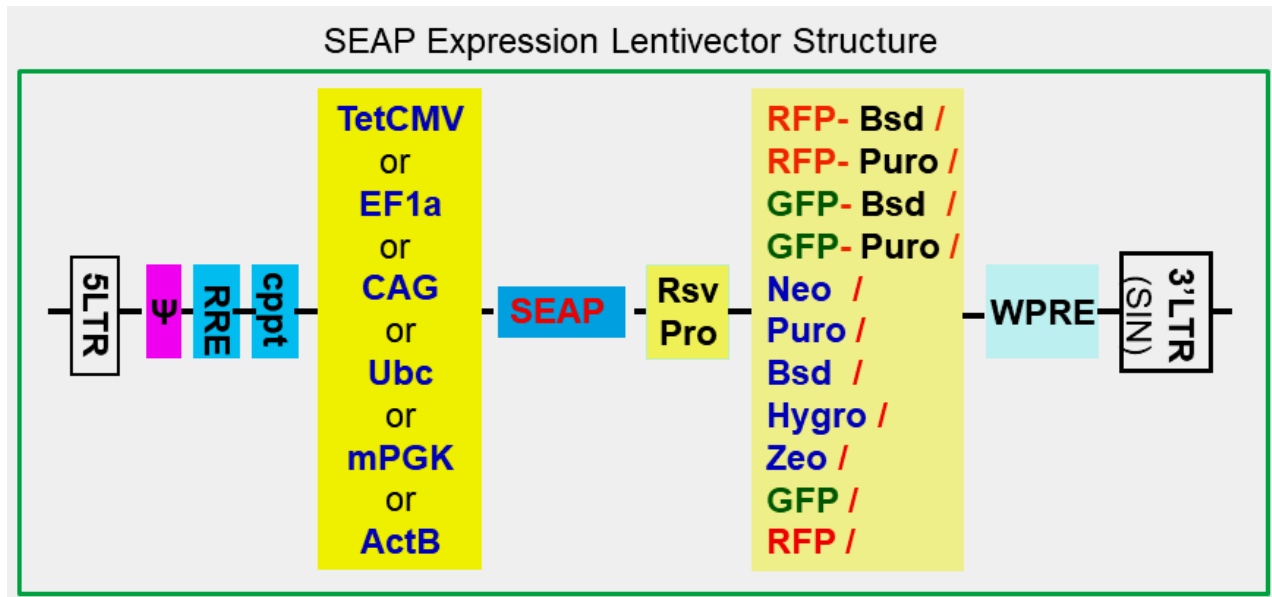
The SEAP is commonly used as a powerful reporter gene for the investigation of promoter activity in transfected eukaryotic cells. The SEAP expression level (reflect its promoter activity) can be detected via chemiluminescent reporter assay based on dioxetane CSPD (chloro-5-substituted adamantyl-1,2-dioxetane phosphate), and provides a convenient and highly sensitive method for the quantitation of transcriptional activity.

The chemiluminescent substrate CSPD is dephosphorylated by alkaline phosphatase (AP), resulting in an unstable dioxetane anion that decomposes and emits light. The light emission has maximal activity at a wavelength of **477nm**. The light signal is quantified in a tube or microplate luminometer. The signal may also be measured in a scintillation counter (single photon mode). The expressed SEAP is stable at 65°C, therefore, you can eliminate the endogenous alkaline phosphatase by heat-inactivation step prior to assaying the reporter gene.

## Premade SEAP expression lentivirus:



GenTarget provides Pre-made lentivirus expressing SEAP under different promoters (**TetCMV**, **EF1a**, **CAG**, or a **signal pathway responsive promoter**), containing an **antibiotic** selection, or a **fluorescent** marker, or **fluorescent-antibiotic fusion** dual selection. See the lentivector's core structure in the following scheme:



## Product Features:

### 1. High expression level under different promoters:

The SEAP was expressed under different promoters. The **TetCMV** promoter is an engineered with highest promoter strength in most cell types. It is also an [optional inducible promoter](#) (click to see details). It constitutively express SEAP in high level without need for any induction. However, optionally, it can be used as inducible expression when its repressor ([TetR](#)) is present. The engineered **EF1a** promoter has medium to high expression in almost all cell types and does not be silenced over long-term cell culture. The **CAG** promoter is a combination of the cytomegalovirus (CMV) early enhancer element and chicken beta-actin promoter. The research showed CAG promoter is more tissue specific promoter, and very active in some types of cells like Embryonic stem cells (ES cells). The **Ubc**, **mPGK**, or **ActB** (actin beta) promoter can be selected at your desirable for the lentivirus application in your specific cell types. **Lentivirus in PBS** are the concentrated lentivirus with higher titer, best suite for serum-free cell culture and for hard-to-infected cell types (like primary cells).



## 2. **Flexible selection of transduced cells:**

To satisfy different antibiotic selection need, Gentarget's expression lentivirus contain an antibiotic marker: Blasticidin (**Bsd**), Puromycin (**Puro**), Neomycin (**Neo**), Hygromycin (**Hygro**), Zeocin (**Zeo**), or a Fluorescent-Antibiotic fusion dual selection: **RFP-Puro**, **RFP-Bsd**, **GFP-Puro**, **GFP-Bsd**. So the positive transduced cells can be selected via either sorting upon fluorescent signal, or antibiotic killing.

## 3. **Easy to use:**

The premade lentivirus was premixed with 10x of polybrene (60 ug/ml) to increase the transduction efficiency. It is very easy to use, simply add 50 ul virus into one well (containing 0.5 ml medium) in 24 well-plate, leave virus on for 48-hours to 72-hours depend upon cell types, then the cells are ready for selection or assay.

## **Ready-to-use lentivirus are provided in two formats:**

- 1) Provided 200ul in 10% of FBS in DMEM and 60ug/ml of polybrene (10x);
- 2) Provided 200ul in PBS solution with concentrated lentivirus. The lentivirus in PBS solution is best for any cell types that requires non-serum in culture medium, and for the hard-to-infected cell types.

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

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.

## **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.



## 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 0.5ml x (half million cells) in a well of a 24-well plate.

## Day 1:

- Remove the culture medium and add 0.5ml fresh, warm, complete medium.
- 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<sub>2</sub> incubator.

**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 ~72hr 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, and select for antibiotic resistance. 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<sub>2</sub> incubator if necessary.

Measure cell density. When density has reached  $\sim 3 \times 10^6$  cells/ml, measured viability should be > 90%. Dilute cells into  $1 \times 10^6$  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  $\mu$ l 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 CO<sub>2</sub> incubator.

## Day 2:



At 24 hours after transduction, add an equal amount of fresh medium containing relevant antibiotics. **Note:** amount of antibiotic depends on cell type. Continue growing cells in CO2 incubator.

### Day 3:

At 72 hours after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

### 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;		

### 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 at handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

### References:

1. BioTechniques 38:891-894(June 2005);
2. THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 279, No. 5, Issue of January 30, pp. 3212-3217, 2004;
3. Biosci. Biotechnol. Biochem., 68(3), 565-5570, 2004;
4. Annu Rev Microbiol. 1994;48:345-69.
5. Microbiol Mol Biol Rev. 2005 Jun;69(2):326-56.
6. APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 2005, p. 3427-3432;
7. Molecular & Biochemical Parasitology 155 (2007) 167-171;
8. 1. Biosci. Biotechnol. Biochem., 68(3), 565-570, 2004;
9. NIH Guidelines for [Biosafety Considerations for Research with Lentiviral Vectors](#). (Link).
10. [CDC guidelines for Lab Biosafety levels](#) (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 product categories.

<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
<a href="#">Pathway Reporter</a>	Repoter Lentivirus for all kinds of pathway screening assays
<a href="#">Cell Immortalization</a>	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.
<a href="#">ImmunoOncology Research</a>	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;
<a href="#">CAR-T, TCR Lentivirus</a>	<b>CARs</b> Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; <b>TCRs</b> : MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRαV3-F2A-TRβV5-6;
<a href="#">CRISPR Gene Editing</a>	Preamde lentivirus express humanized wild-type <b>Cas9</b> endonuclease, the <b>dCas9</b> , gRNAs, <b>CRISPR</b> gene editing research
<a href="#">Epigenomic: CRISPRi and CRISPRa</a>	" <b>dCas9-Protein</b> " fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).
<a href="#">Cell-Specific Reporter</a>	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
<a href="#">Infectious Antigens</a>	Lentivirus that express all kinds of infectious antigens with C-term 6His-tag.
<a href="#">Virus Like Particles (VLP)</a>	Lentiviral Like Particles, pseudo-typed with a different envelope proteins.
<a href="#">Non-integrating LV</a>	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.
<a href="#">shRNA Knockdown</a>	Knockdown verified and customized shRNA lentivirus for target knockdown,



<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
<a href="#">microRNA lentivirus</a>	Premade lentivirus expression human or mouse <b>precursor miRNA</b> . And <b>anti-miRNA</b> lentivector and virus for human and mouse miRNA.
<a href="#">Anti-miNA lentivirus</a>	Pre-made lentivirus expression a specific anti-miRNA cassette.
<a href="#">Human and mouse ORFs</a>	Premade lentivirus expressin a <b>human, mouse or rat</b> gene with RFP-Blastididin fusion dual markers.
<a href="#">Luciferase expression</a>	Premade lentivirus for all kinds of luciferase protein expression: <b>firefly and Renilla, Red-Luc and more</b> , with different antibiotic selection markers.
<a href="#">Fluorescent Markers</a>	Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, niRFP, unstable GFP and others.
<a href="#">Luminescent Imaging</a>	Lentivirus express Nano-Latern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms
<a href="#">Sub-cellular Imaging</a>	Lentivirus contain a well-defined organelle targeting signal fused to a fluorescent protein, great tools for live-cell imaging and for dynamic investigation of sub-cellular signal pathways.
<a href="#">Cytoskeleton Imaging</a>	A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure
<a href="#">Unstable GFP</a>	Lentivirus express the the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway assay and in knockdown / knockout detection
<a href="#">near-infrared RFP</a>	The near-infrared Red fluorescent (niRFP) expression Lentiviurs provides the whole-body images with better contrast and brighter images
<a href="#">Fluorescent-ORF fusion</a>	Pre-made lentivirus expression a " <b>GFP/RFP/CFP-ORF</b> " fusion target.
<a href="#">CRE recombinase</a>	Premade lentivirus for expressing <b>nuclear permeant CRE</b> recombinase with different flurescent and antibiotic markers.
<a href="#">CRE, Flp ColorSwitch</a>	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.



<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
<a href="#">SEAP Reporter</a>	lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter),
<a href="#">TetR Repressor</a>	Premade lentivirus expressing TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
<a href="#">rtTA Expression</a>	rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF inducible system.
<a href="#">iPS factors</a>	Premade lentivirus for human and mouse iPS ( <b>Myc, NANOG, OCT4, SOX2, FGF4</b> ) factors with different fluorescent and antibiotic markers
<a href="#">LacZ expression</a>	Express different full length <b><math>\beta</math>-galactosidase (lacZ)</b> with different selection markers
<a href="#">Negative control lentiviruses</a>	Premade <b>negative control lentivirus with different markers</b> : serves as the negative control of lentivirus treatment, for validation of the specificity of any lentivirus target expression effects.
<a href="#">Other Enzyme expression</a>	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.
<a href="#">Ultra titer lentivirus</a>	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.