



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

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



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



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



LVP1220-PBS	SEAP (mPGK, Puro) lentivirus in PBS	
LVP1221-PBS	SEAP (ActB, Puro) 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.

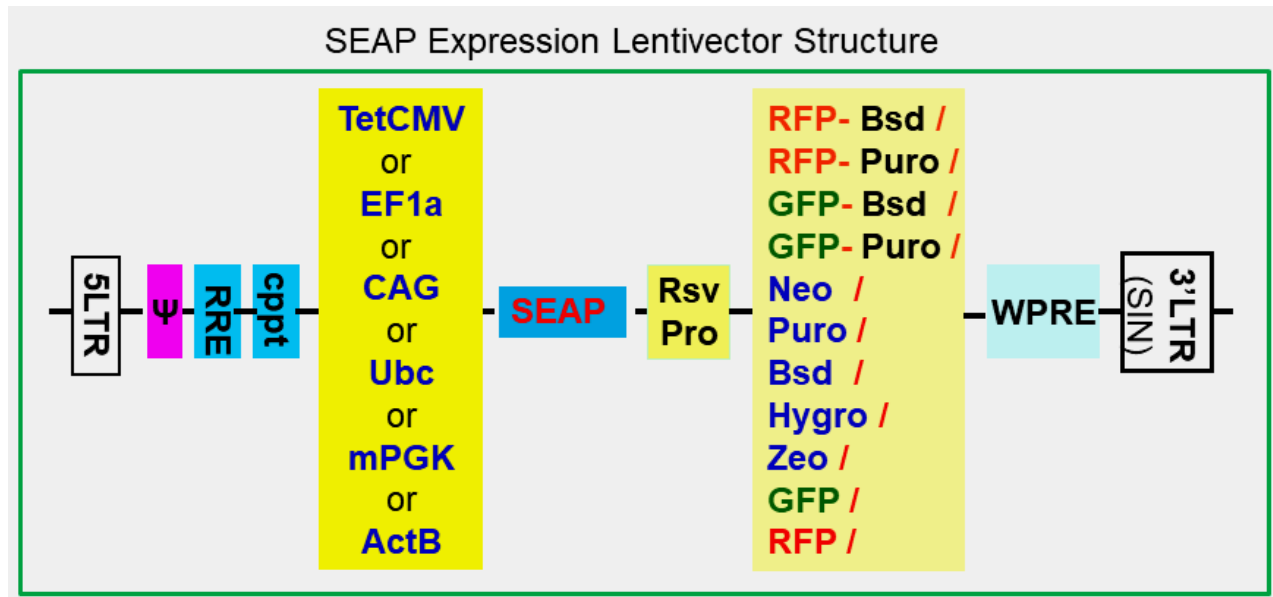
The SEAP is commonly used as a powerful reporter gene for the investigation of promoter activity in transfected eukaryotic cells. The SEAP expression level (reflect its promoter activity) can be detected via chemiluminescent reporter assay based on dioxetane CSPD (chloro-5-substituted adamantyl-1,2-dioxetane 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, CO₂ 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 CO₂ incubator if necessary.

Measure cell density. When density has reached ~3 x 10⁶ cells/ml, measured viability should be > 90%. Dilute cells into 1 x 10⁶ 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.

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.

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.

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;		



Related Products: GenTarget's pre-made lentivirus product category.

Product Category	Product Description (please click category name to see product's pages)
Human, mouse or rat ORFs	Premade lentivirus expressing a human, mouse or rat gene with RFP-Blastididin fusion dual markers.
Fluorescent markers	Premade lentivirus express human codon optimized fluorescent protein, GFP / RFP / CFP / BFP / YFP .
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression: firefly and Renilla with different antibiotic selection markers.
CRE recombinase	Premade lentivirus for expressing nuclear permeant CRE recombinase with different fluorescent and antibiotic markers.
LoxP ColorSwitch	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
CRISPR /hu CAS9	Premade lentivirus express humanized wild-type Cas9 endonuclease for genomic editing with CRISPR
TetR inducible expression repressor	Premade lentivirus expressing TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
iPS factors	Premade lentivirus for human and mouse iPS (Myc, NANOG, OCT4, SOX2, FLK4) factors with different fluorescent and antibiotic markers
T-antigen Expression	Express SV40 large T antigen with different selection markers
Cell Organelle imaging	Premade lentivirus for cell organelle imaging. The fluorescent marker GFP/RFP/CFP was sub-cellular localized in different cell organelle for living cell imaging.
LacZ expression	Express different full length β-galactosidase (lacZ) with different selection markers
Anti-miRNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.
Fluorescent-ORF fusion	Pre-made lentivirus expression a " GFP/RFP/CFP-ORF " fusion target.
Pre-made shRNA lentivirus	Premade shRNA lentivirus for knockdown a specific genes (P53, LacZ, Luciferase and more).
microRNA and anti-	Premade lentivirus expression human or mouse precursor miRNA . And anti-miRNA lentivector and virus for human and



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microRNA lentivirus	mouse miRNA.
Negative control lentiviruses	Premade negative control lentivirus with different markers : serves as the negative control of lentiviruses treatment, for validation of the specificity of any lentivirus target expression effects.
Other Enzyme	Ready-to-use lentivirus, expressing specific enzymes with different selection markers.