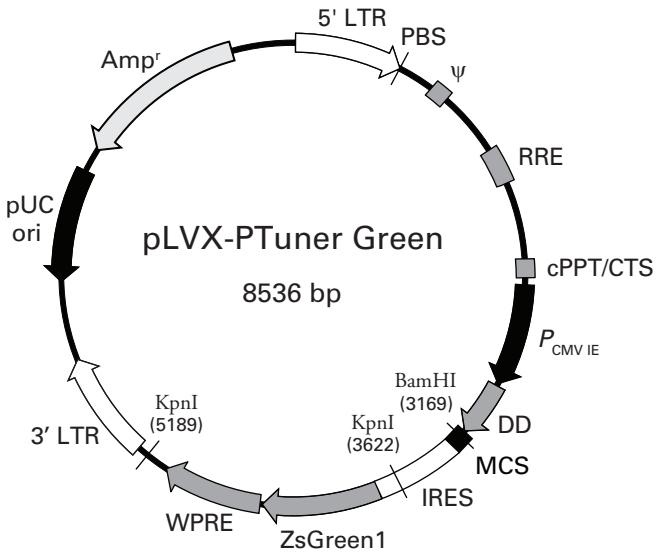
Vector Information

pLVX-PTuner Green Vector Information PT4054-5

Catalog No. 632175



	End of DD-tag		AgeI		EcoRI		XhoI		SpeI		XbaI		NotI			BamHI	
3121	AAA	CCG	GAA	ACC	GGT	GAA	TTC	CTC	GAG	ACT	AGT	TCT	AGA	GCG	GCC	GCG	GAT	CCC

pLVX-PTuner Green Vector Map and Multiple Cloning Site (MCS).

Description

pLVX-PTuner Green is a bicistronic, HIV-1-based, lentiviral expression vector that allows you to precisely regulate the amount of your protein of interest in virtually any mammalian cell type, including primary cells. The vector encodes a 12 kDa, FKBP (L106P) destabilization domain (DD; 1) that is expressed as an N-terminal tag on your protein of interest, causing rapid degradation of the fusion protein. Degradation of the DD-tagged protein can be prevented by the addition of Shield1 stabilizing ligand to the medium. Shield1 is a membrane permeable molecule that binds to the DD tag, 'shielding' the fusion protein from proteasomal degradation.

pLVX-PTuner Green allows the simultaneous expression of your DD-tagged protein of interest and the fluorescent protein ZsGreen1 from the same bicistronic mRNA transcript. ZsGreen1 is a human codon-optimized variant of the reef coral *Zoanthus sp.* green fluorescent protein, ZsGreen (2). Expression of the bicistronic transcript is driven by the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$) located just upstream of the DD tag coding sequence. An encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES), positioned between the MCS and the ZsGreen1 gene (see above), facilitates cap-independent translation of ZsGreen1 from an internal start site at the IRES/ZsGreen1 junction (3). Because ZsGreen1 is unaffected by the DD tag, it can be used as an indicator of transfection and transduction efficiency, as well as a marker for selection and cell sorting.

pLVX-PTuner Green contains all of the viral processing elements necessary for the production of replication-incompetent lentivirus, as well as elements to improve viral titer, transgene expression, and overall vector function. The woodchuck hepatitis virus posttranscriptional

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regulatory element (WPRE) promotes RNA processing events and enhances nuclear export of viral and transgene RNA (4), leading to increased viral titers from packaging cells and enhanced expression of your gene of interest in target cells. In addition, the vector includes a Rev-response element (RRE), which further increases viral titers by enhancing the transport of unspliced viral RNA out of the nucleus (5). Finally, pLVX-PTuner Green also contains a central polypurine tract/central termination sequence element (cPPT/CTS). During target cell infection, this element creates a central DNA flap that increases nuclear import of the viral genome, resulting in improved vector integration and more efficient transduction (6). The vector also contains a pUC origin of replication and an *E. coli* ampicillin resistance gene (Amp^r) for propagation and selection in bacteria.

Use

pLVX-PTuner Green is available in the Lenti-X™ ProteoTuner™ Shield System N (w/ ZsGreen1) [Cat. No. 632175]. The vector is designed to constitutively coexpress a DD-tagged protein of interest and ZsGreen1 from $P_{CMV\ IE}$ when transduced into mammalian cells. In order to create your N-terminally tagged protein of interest, your gene of interest must be cloned into the MCS in the same reading frame as the DD tag sequence, and it must contain a stop codon at the end of its coding sequence. Before it can be transduced into target cells, the vector must be cotransfected into 293T cells with our Lenti-X™ HTX Packaging System (Cat. Nos. 631247 and 631249) and packaged into viral particles. This packaging system allows the safe production of high titer, infectious, replication-incompetent, VSV-G pseudotyped lentiviral particles that can infect a wide range of cell types, including non-dividing and primary cells (7).

ZsGreen1 is the brightest commercially available green fluorescent protein. The presence of this protein allows the selection of stable transductants by flow cytometry (or other detection methods) with standard FITC filter sets (ZsGreen1 has an excitation maximum of 493 nm and an emission maximum of 505 nm).

When cells expressing a DD-tagged protein of interest are grown in medium containing Shield1, the ligand binds to the DD tag and protects the fusion protein from degradation. As a result, the protein quickly accumulates inside the cells in amounts that are directly proportional to the concentration of Shield1 in the medium. If the cells are subsequently grown in medium lacking Shield1, the DD tag is no longer stabilized, and the fusion protein is rapidly degraded. Because the effects of Shield1 are concentration-dependent and reversible, it is possible to fine-tune the amount of fusion protein present in the cells simply by adjusting the concentration of the ligand in the medium (1).

Location of Features

- 5' LTR: 1–635
- PBS (primer binding site): 636–653
- Ψ (packaging signal): 685–822
- RRE (Rev-response element): 1303–1536
- cPPT/CTS (central polypurine tract/central termination sequence): 2028–2151
- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 2185–2788
- DD (FKBP-L106P destabilization domain): 2806–3129
- MCS (multiple cloning site): 3131–3169
- IRES (encephalomyocarditis virus internal ribosome entry site): 3175–3749
- ZsGreen1(*Zoanthus sp.* green fluorescent protein): 3750–4445
- WPRE (woodchuck posttranscriptional regulatory element): 4459–5050
- 3' LTR: 5254–5890
- pUC origin of replication: 6360–7030 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 7175–8171 (complementary)

Selection of Stable Transfectants

- Selectable marker: ZsGreen1.

Propagation in *E. coli*

- Suitable host strains: Stellar™ Competent Cells.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

Notes:

The vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.

The viral supernatants produced by this lentiviral vector could contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant lentivirus. Appropriate NIH, regional, and institutional guidelines apply.

References

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