Certificate of Analysis

Lenti-X™ DD-AmCyan1 Vector Set

Catalog No.	Amount	Lot Number
631749 (Not sold separately) Sold as a part of 631748	Each	Specified on product label.

Product Information

The Lenti-X DD-AmCyan1 Vector Set (available as part of the Lenti-X™ DD Cyan Reporter System; Cat. No. 631748) includes two HIV-1-based, lentiviral expression vectors that can efficiently transduce both dividing and nondividing mammalian cells. This reporter set can be used to monitor promoter activity in live cells and *in vivo.*

- pLVX-DD-AmCyan1 Reporter is a promoterless vector that can be used to monitor transcription from different promoters and promoter/enhancer combinations inserted into the multiple cloning site (MCS). The gene downstream of the MCS encodes the cyan fluorescent protein DD-AmCyan1, a modified version of AmCyan1 that is tagged on its N-terminus with the ProteoTuner™ destabilization domain (DD; Cell, 2006). In the absence of the Shield1 ligand, the DD tag induces rapid degradation of the fluorescent reporter, minimizing any background caused by leaky promoters; but upon addition of Shield1 at the time of promoter activation, the DD-tagged reporter molecules are stabilized, increasing the signal-to-noise ratio.
- pLVX-DD-AmCyan1 Control drives reporter expression via a constitutive promoter, and thereby serves as a positive control.

DD-AmCyan1 Reporter

AmCyan1 (excitation and emission maxima: 453 and 486, respectively) is a human codon-optimized variant of the wild-type *Anemonia majano* cyan fluorescent protein (AmCyan) that exhibits enhanced emission characteristics (Nature Biotcech., 1999; Curr. Biol., 1996). DD-AmCyan1 is tagged on its N-terminus with the ProteoTuner DD, which causes rapid, proteasomal degradation of DD-AmCyan1. However, when the membrane-permeant, stabilizing ligand Shield1 is added to the medium, it binds to the DD and prevents degradation of the DD-AmCyan1 reporter protein, thereby causing it to accumulate inside the cell.

In the absence of the stabilizing ligand Shield1, the DD causes the degradation of any DD-AmCyan1 reporter protein produced prior to promoter activation, thus minimizing background fluorescence caused by leaky promoters. To analyze promoter activity, the inducer of choice is added to the medium along with Shield1, which effectively stabilizes the reporter protein, allowing it to accumulate. As a result, only the reporter molecules expressed during promoter induction will contribute to the fluorescence signal, providing a considerably higher signal-to-noise ratio than that obtained with non-destabilized or constitutively destabilized reporter systems.

For both vectors, the promoter's activity level can be directly correlated to the fluorescence level.

Lentiviral Elements

The reporter and control vectors each contain 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 regulatory element (WPRE) promotes RNA processing events and enhances nuclear export of viral and transgene RNA (J. Virol, 1999), leading to increased viral titers from packaging

cells, and enhanced expression of your gene of interest in target cells. In addition, each vector includes a Rev-response element (RRE), which further increases viral titers by enhancing the transport of unspliced viral RNA out of the nucleus (Proc. Natl. Acad. Sci. USA, 1990). Finally, each vector 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 (Cell, 2000).

Lentiviral particles derived from the vectors allow you to monitor your promoter of interest in virtually any cell type, even primary cells.

Antibiotic Selection

In addition to lentiviral elements, the reporter and control vectors each contain a puromycin resistance gene (Puro^r) under the control of the murine phosphoglycerate kinase promoter (P_{PGK}) for the selection of stable transductants. The vectors also contain pUC origins of replication and *E. coli* ampicillin resistance genes (Amp^r) for propagation and selection in bacteria.

Package Contents

- 20 µg pLVX-DD-AmCyan1 Reporter Vector
- 20 µg pLVX-DD-AmCyan1 Control Vector

Storage Conditions

- Store at –20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Storage Buffer

- 10 mM Tris-HCl (pH 8.0)
- 1 mM EDTA (pH 8.0)

Concentration

- 500 ng/µl

Shipping Conditions

- Dry ice

Product Documents

Documents for our products are available for download at **takarabio.com/manuals**
The following documents apply to this product:

- DD-Fluorescent Protein Reporter Systems Protocol-At-A-Glance
- Lenti-X Lentiviral Expression Systems User Manual

pLVX-DD-AmCyan1 Reporter Vector

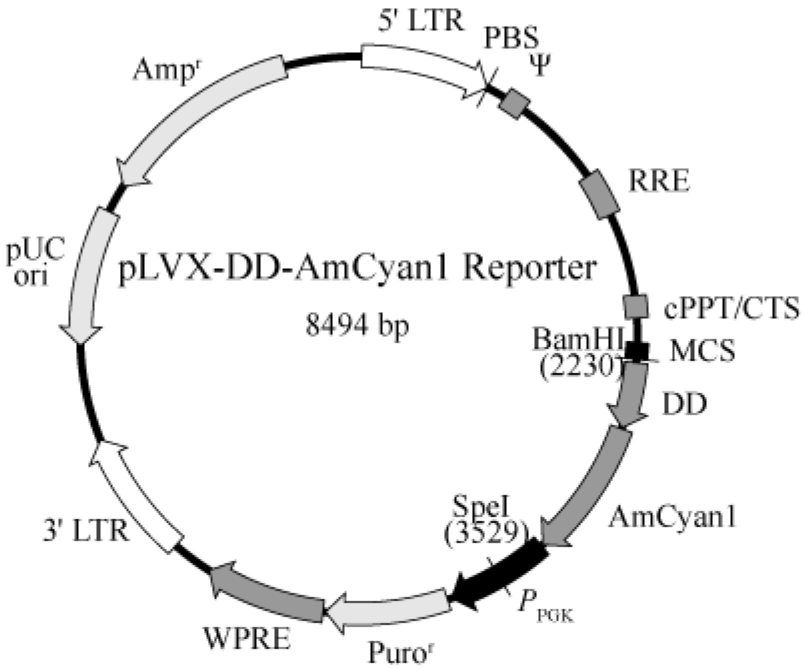

Figure 1. pLVX-DD-AmCyan1 Reporter vector map

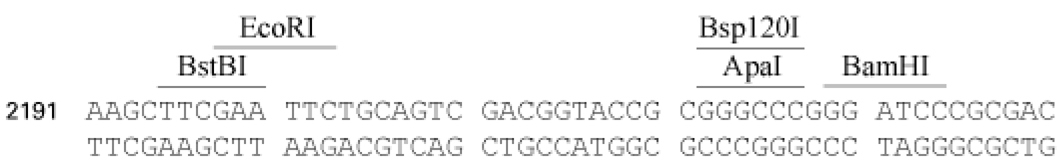

Figure 2. pLVX-DD-AmCyan1 Reporter vector multiple cloning site

Description

pLVX-DD-AmCyan1 Reporter is a promoterless reporter vector that allows the functional analysis of different promoters and promoter/enhancer combinations inserted into its multiple cloning site (MCS). The vector encodes the reporter protein DD-AmCyan1, a ligand-dependent, destabilized cyan fluorescent protein that minimizes background fluorescence from leaky promoters. A promoter must be cloned into the MCS, located upstream of the DD-AmCyan1 coding sequence. Without the addition of a functional promoter, the vector will not express DD-AmCyan1.

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
- MCS (multiple cloning site): 2195–2234
- DD (FKBP-L106P destabilization domain): 2247–2570
- AmCyan1 (*Anemonia majano* cyan fluorescent protein): 2577–3263
- P_{PGK} (phosphoglycerate kinase promoter): 3274–3782

- Puro^r (puromycin resistance gene): 3803–4402
- WPRE (woodchuck hepatitis virus posttranscriptional regulatory element): 4416–5007
- 3' LTR: 5211–5847
- pUC origin of replication: 6317–6987 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 7132–8128 (complementary)

pLVX-DD-AmCyan1 Control Vector

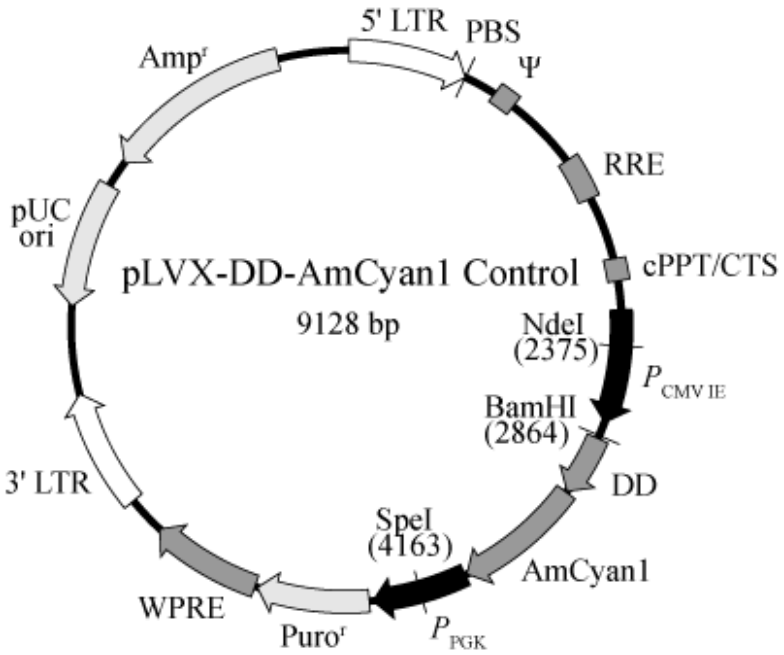

Figure 3. pLVX-DD-AmCyan1 Control vector map

Description

pLVX-DD-AmCyan1 Control constitutively expresses the destabilized cyan fluorescent protein DD-AmCyan1. The vector can be used as a control to monitor ligand-dependent stabilization of DD-AmCyan1 in your cell-type of interest.

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): 2881–3204
- AmCyan1 (*Anemonia majano* cyan fluorescent protein): 3211–3897
- P_{PGK} (phosphoglycerate kinase promoter): 3908–4416
- Puro^r (puromycin resistance gene): 4437–5036
- WPRE (woodchuck hepatitis virus posttranscriptional regulatory element): 5050–5641
- 3' LTR: 5845–6481
- pUC origin of replication: 6951–7621 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 7766–8762 (complementary)

Additional Information

Propagation in *E. coli*

- Recommended host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

Excitation and Emission Maxima of AmCyan1

- Excitation: 453 nm
- Emission: 486 nm

References

Banaszynski, L. A., Chen, L.-C., Maynard-Smith, L. A., Ooi, A. G. L. & Wandless, T. J. A rapid, reversible, and tunable method to regulate protein function in living cells using synthetic small molecules. *Cell* **126,** 995–1004 (2006).

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Matz, M. V. *et al.* Fluorescent proteins from nonbioluminescent Anthozoa species. *Nat. Biotechnol.* **17,** 969–973 (1999).

Zennou, V. *et al.* HIV-1 genome nuclear import is mediated by a central DNA flap. *Cell* **101,** 173–85 (2000).

Zufferey, R., Donello, J. E., Trono, D. & Hope, T. J. Woodchuck hepatitis virus posttranscriptional regulatory element enhances expression of transgenes delivered by retroviral vectors. *J. Virol.* **73,** 2886–92 (1999).

Quality Control Data

Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Vector	Enzymes	Fragments (kb)
pLVX-DD-AmCyan Reporter	BamHI and SpeI	1.3 & 7.2
pLVX-DD-AmCyan Control	NdeI and SpeI	1.8 & 7.3

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280}: 1.8–2.0

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

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

Notice to Purchaser

Lenti-X™ DD-AmCyan1 Vector Set

CATALOG NO.

631749

NOTICE TO PURCHASER:

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

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STATEMENT 57

This product is covered by U.S. Patent Nos. 8,173,792 and 9,487,787.

TRADEMARKS:

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