Certificate of Analysis

Lenti-X™ DD-tdTomato Vector Set

Catalog No.	Amount	Lot Number
631754 (Not sold separately) Sold as a part of 631753	Each	Specified on product label.

Product Information

The Lenti-X DD-tdTomato Vector Set 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-tdTomato 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 red fluorescent protein DD-tdTomato, a modified version of TdTomato 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-tdTomato Control drives reporter expression via a constitutive promoter, and thereby serves as a positive control.

DD-tdTomato Reporter

tdTomato (excitation and emission maxima: 554 and 581 nm, respectively) is a member of the family of fruit fluorescent proteins derived from the *Discosoma* sp. red fluorescent protein, DsRed (Nature Biotech, 2004). The vector was designed with two copies of the Tomato coding region linked together to allow intramolecular dimerization. As a result, each tdTomato RNA transcript encodes a tandem dimer of the Tomato protein (Proc. Natl. Acad. Sci. USA, 2002).

DD-tdTomato is tagged on its N-terminus with the ProteoTuner DD, which causes rapid, proteasomal degradation of DD-tdTomato. However, when the membrane-permeant, stabilizing ligand Shield1 is added to the medium, it binds to the DD and prevents degradation of the DD-tdTomato 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-tdTomato 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 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-tdTomato Reporter Vector
- 20 µg pLVX-DD-tdTomato 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-tdTomato Reporter Vector Information
- pLVX-DD-tdTomato Control Vector 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 tdTomato

- Excitation: 554 nm
- Emission: 581 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).

Campbell, R. E. *et al.* A monomeric red fluorescent protein. *Proc. Natl. Acad. Sci. U. S. A.* **99,** 7877–7882 (2002).

Cochrane, A. W., Chen, C. H. & Rosen, C. A. Specific interaction of the human immunodeficiency virus Rev protein with a structured region in the env mRNA. *Proc. Natl. Acad. Sci. U. S. A.* **87,** 1198–1202 (1990).

Shaner, N. C. *et al.* Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nat. Biotechnol.* **22,** 1567–72 (2004).

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-tdTomato Reporter	BamHI and SpeI	2.0 & 7.2
pLVX-DD-tdTomato Control	NdeI and NotI	2.3 & 7.6

- 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

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CATALOG NO.

631754

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 44

The DsRed-Monomer and the Fruit Fluorescent Proteins are covered by one or more of the following U.S. Patents: 7,671,185, 7,9110,714 and 8,664,471.

STATEMENT 57

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

STATEMENT 72

Living Colors Fluorescent Protein Products:

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Takara Bio USA, Inc.
2560 Orchard Parkway, San Jose, CA 95131, USA
U.S. Technical Support: technical_support@takarabio.com

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.565.6999

9/12/2023

Notice to Purchaser

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STATEMENT 69

The DsRed-Monomer, DsRed Express, E2-Crimson and the Fruit Fluorescent Proteins are covered by one or more of the following U.S. Patents: 7,250,298; 7,671,185; 7,910,714; 8,664,471 and 8,679,749.

TRADEMARKS:

Takara Bio USA, Inc.
2560 Orchard Parkway, San Jose, CA 95131, USA
U.S. Technical Support: technical_support@takarabio.com

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.565.6999

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