Certificate of Analysis

pDD-tdTomato Reporter

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Catalog No.	Amount	Lot Number
632193 (Not sold separately)	20 µg	Specified on product label.

Product Information

pDD-tdTomato Reporter is sold as part of the DD-tdTomato Reporter System (Cat. No. 632190). pDD-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 red fluorescent protein tdTomato, tagged at its N-terminus with the ProteoTuner™ destabilization domain (DD; 1). 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.

Package Contents

- 20 µg pDD-tdTomato Reporter

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 (–70°C)

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Product User Manuals

User manuals for Clontech products are available for download at www.clontech.com/manuals.
The following user manuals apply to this product:

- DD-Fluorescent Protein Reporter Systems Protocol-At-A-Glance (PT4088-2)
- ProteoTuner Systems User Manual (PT4039-1)
- pDD-tdTomato Reporter Vector Information

Description

pDD-tdTomato 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-tdTomato, a ligand-dependent, destabilized red fluorescent protein that minimizes background fluorescence from leaky promoters. This reporter can be used to monitor promoter activity in live cells and *in vivo*. A promoter must be cloned into the MCS, located upstream of the DD-tdTomato coding sequence. Without the addition of a functional promoter, the vector will not express DD-tdTomato.

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 (2). 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 (3).

DD-tdTomato is a modified version of tdTomato that is tagged on its N-terminus with the ProteoTuner DD, which causes rapid, proteasomal degradation of DD-tdTomato (1). 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 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.

The promoter's activity level can be directly correlated to the fluorescence level.

Vector Elements

The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. pDD-tdTomato can be transfected into mammalian cells using any standard transfection method.

Antibiotic Selection

A neomycin-resistance cassette (Neo^r) allows stably transfected eukaryotic cells to be selected using G418 (4). This cassette consists of the SV40 early promoter, a Tn5 kanamycin/neomycin resistance gene, and herpes simplex virus thymidine kinase (HSV TK) polyadenylation signals. A bacterial promoter upstream of the cassette expresses kanamycin resistance in *E. coli*.

Additional Information

Propagation in *E. coli*

- Recommended host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high
- Plasmid incompatibility group: pMB1/ColE1

Excitation and Emission Maxima of tdTomato

- Excitation: 554 nm
- Emission: 581 nm

References

1. Banaszynski, L. *et al*. (2006) *Cell* **126**(5):995–1004.
2. Shaner, N. C., *et al*. (2004) *Nature Biotech*. **22**(12):1567-1572.
3. Campbell, R. E. *et al*. (2002) *Proc. Natl. Acad. Sci. USA* **99**(12):7877–7882.
4. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II.* Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143–190.

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:

Enzymes	Fragments (kb)
BamHI	5.4
AgeI & NotI	1.8 & 3.6

- 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 Clontech. This vector has not been completely sequenced.

Notice to Purchaser

pDD-tdTomato Reporter

CATALOG NO.

632193

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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: 7,005,511; 7,157,566; 7,393,923 and 7,250,298.

STATEMENT 57

Patent Pending

STATEMENT 72

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