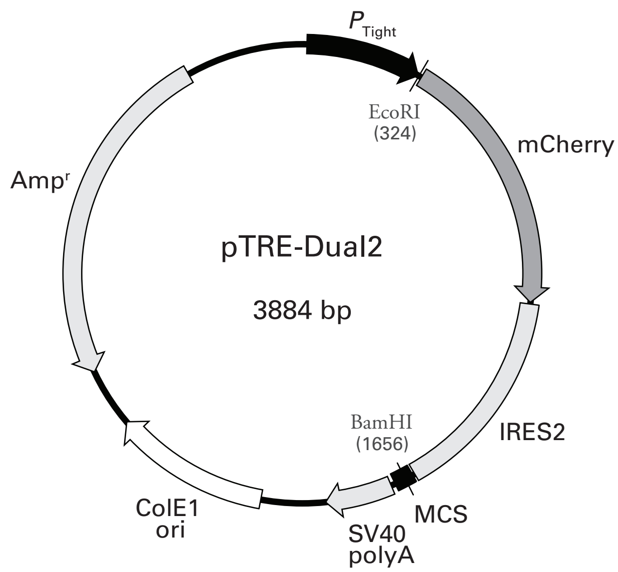
Vector Information

Sold as part of Catalog Nos. 631112 and 631113




```
                                    NotI
       End of IRES2                 EagI      BglII     BamHI     ClaI      SalI
1628   ATG GCC ACA ACC GCG GCC GCT AGA TCT GGA TCC ATC GAT GTC GAC
       TAC CGG TGT TGG CGC CGG CGA TCT AGA CCT AGG TAG CTA CAG CTG

       EcoRV    NdeI     XbaI
1673   GAT ATC CAT ATG TCT AGA
       CTA TAG GTA TAC AGA TCT
```

pTRE-Dual2 Vector Map and Multiple Cloning Site (MCS). The internal start site (ATG) at the IRES2/MCS junction is indicated in bold.

Description

pTRE-Dual2 is a tetracycline (Tet)-regulatable, mammalian expression vector designed to coexpress a gene of interest and the red fluorescent protein mCherry under the control of P_{Tight}, a modified Tet-responsive promoter. P_{Tight} consists of a modified minimal CMV promoter, and seven direct repeats of a 36 bp regulatory sequence that contains the 19 bp tet operator sequence (*tetO*; 1). This vector is designed to be used with our Tet-On® Advanced and Tet-Off® Advanced Inducible Gene Expression Systems (Cat. Nos. 630930 and 630934). These systems provide the inducible gene expression strategy of Gossen & Bujard, with major improvements described by Urlinger, *et al.* (2–6).

mCherry is a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (excitation and emission maxima: 587 nm and 610 nm, respectively; 7). pTRE-Dual2 coexpresses mCherry and a gene of interest (cloned into the multiple cloning site [MCS]) from a bicistronic mRNA transcript, in a doxycycline (Dox) controllable manner. An encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES2), positioned between mCherry and the gene of interest, facilitates cap-independent translation of the gene of interest from an internal start site at the IRES2/MCS junction (8). This ensures that a high percentage of mCherry-expressing clones also express the gene of interest, allowing mCherry to be used as an indicator of inducibility and transfection efficiency, as well as a marker for selection by flow cytometry. The vector also contains a ColE1 origin of replication and an ampicillin resistance gene (Amp^r) to allow for propagation and selection in *E. coli*.

(PR093634; published 7 September 2010)

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Use

pTRE-Dual2 is a mammalian expression vector that allows tightly regulated, doxycycline-controlled coexpression of mCherry and a protein of interest. The gene of interest must have both a start and a stop codon. For enhanced expression, the gene should also be cloned in-frame with the start codon at the IRES2/MCS junction (this codon is shown in bold in the MCS sequence on page 1).

In order to function, the system requires the presence of a tetracycline-controlled transcriptional activator (Tet-On Advanced or Tet-Off), supplied by a stable Tet-On Advanced or Tet-Off Advanced cell line that can be created with our Tet-On Advanced or Tet-Off Advanced Inducible Gene Expression Systems (Cat. Nos. 630930 and 630934).

Coexpression of mCherry and the gene of interest allows the use of fluorescence microscopy or flow cytometry to easily monitor and/or select cells expressing the gene of interest (mCherry has an excitation maximum of 587 nm and an emission maximum of 610 nm).

Location of features

- P_{Tight} (modified Tet-responsive promoter): 8–321
- mCherry (human codon-optimized): 335–1045
- IRES2 (encephalomyocarditis virus internal ribosome entry site): 1052–1636
- MCS (multiple cloning site 2): 1640–1690
- SV40 polyA signal: 1697–1879
- ColE1 origin of replication: 2055–2479
- Amp^r (ampicillin resistance gene; β-lactamase): 2641–3564 (complementary)

Propagation in *E. coli*

- Recommended host strain: DH5α™, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Plasmid incompatibility group: pMB1/ColE1

Excitation and emission maxima of mCherry

- Excitation maximum = 587 nm
- Emission maximum = 610 nm

References

1. pTRE-Tight Vectors (April 2003) *Clontechniques* **XVIII**(3):13–14.
2. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci USA* **89**(12):5547–5551.
3. Gossen, M., *et al.* (1995) *Science* **268**(5218):1766–1769.
4. Urlinger, S. *et al.* (2000) *Proc. Natl. Acad. Sci. USA* **97**(14):7963–7968.
5. Inducible Gene Expression Systems (January 2007) *Clontechniques* **XXII**(1):1–2.
6. Tet-On Advanced Inducible Gene Expression System (2006) *Clontechniques* **XXI**(2):1–3.
7. Shaner, N. C. *et al.* (2004) *Nature Biotech.* **22**(12):1567-1572.
8. Jang, S. K. *et al.* (1988) *J. Virol.* **62**(8):2636–2643.

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.

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