



pTet-DualON Vector Map.

### Description

pTet-DualON constitutively coexpresses the tetracycline (Tet)-controlled transcriptional activator Tet-On<sup>®</sup> Advanced (1–4) and the green fluorescent protein ZsGreen1. Tet-On Advanced is a fusion of the Tet repressor, and three minimal “F”-type transcriptional activation domains from the herpes simplex virus VP16 protein. The gene encoding Tet-On Advanced is completely synthetic, lacks cryptic splice sites, and utilizes human codon preferences for stable expression in mammalian cells. ZsGreen1 is a human codon-optimized variant of the reef coral *Zoanthus sp.* green fluorescent protein (ZsGreen) that has been engineered for brighter fluorescence (excitation and emission maxima: 493 and 505 nm, respectively; 5, 6).

The vector coexpresses ZsGreen1 and Tet-On Advanced from a bicistronic mRNA transcript. Expression is driven by the powerful, constitutively active human cytomegalovirus immediate early promoter ( $P_{CMV IE}$ ). An encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES2), positioned between Tet-On Advanced and ZsGreen1, facilitates cap-independent translation of ZsGreen1 from an internal start site at the IRES2/ZsGreen1 junction (7). This ensures that a high percentage of ZsGreen1-expressing clones also express Tet-On Advanced, allowing ZsGreen1 to be used as an indicator of transfection efficiency and a marker for selection by flow cytometry. The vector also contains a CoIE1 origin of replication and an ampicillin resistance gene ( $Amp^r$ ) to allow for propagation and selection in *E. coli*.

### Use

pTet-DualON is used to develop stable cell lines that constitutively express Tet-On Advanced. Such cell lines are used in conjunction with a Tet response vector, such as pTRE-Dual2, to establish inducible, doxycycline-controlled gene expression systems, which express a gene of interest under the control of the Tet-responsive element (TRE) in the promoter  $P_{Tight}$  (8).

ZsGreen1 is the brightest commercially available green fluorescent protein. The presence of this protein allows transfectants to be visualized by fluorescence microscopy and sorted by flow cytometry with standard FITC filter sets (ZsGreen1 has an excitation maximum of 493 nm and an emission maximum of 505 nm).

(PR093636; published 7 September 2010)



**Clontech**

United States/Canada  
800.662.2566

Asia Pacific  
+1.650.919.7300

Europe  
+33.(0)1.3904.6880

Japan  
+81.(0)77.543.6116

Clontech Laboratories, Inc.  
A Takara Bio Company  
1290 Terra Bella Ave.  
Mountain View, CA 94043  
Technical Support (US)  
E-mail: tech@clontech.com  
www.clontech.com

**Location of features**

- $P_{CMVIE}$  (human cytomegalovirus immediate early promoter): 7–606
- Tet-On Advanced: 634–1380
- IRES2 (encephalomyocarditis virus internal ribosome entry site): 1389–1973
- ZsGreen1 (*Zoanthus sp.* green fluorescent protein): 1979–2674
- SV40 polyA signal: 2712–2901
- ColE1 origin of replication: 3077–3501
- Amp<sup>r</sup> (ampicillin resistance gene;  $\beta$ -lactamase): 3842–4837 (complementary)

**Propagation in *E. coli***

- Recommended host strain: DH5 $\alpha$ <sup>TM</sup>, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100  $\mu$ g/ml) in *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Plasmid incompatibility group: pMB1/ColE1

**Excitation and emission maxima of ZsGreen1**

- Excitation maximum = 493 nm
- Emission maximum = 505 nm

**References**

1. Tet-On Advanced Inducible Gene Expression System (2006) *Clontechniques XXI*(2):1–3.
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. Matz, M. V. *et al.* (1999) *Nature Biotech.* **17**(10):969–973.
6. Haas, J. *et al.* (1996) *Curr. Biol.* **6**(3):315–324
7. Jang, S. K. *et al.* (1988) *J. Virol.* **62**(8):2636–2643.
- 8 pTRE-Tight Vectors (April 2003) *Clontechniques XVIII*(3):13–14.

**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**

Clontech products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Clontech 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 written approval of Clontech Laboratories, Inc.

DH5 $\alpha$ <sup>TM</sup> is a trademark of Invitrogen Corporation.

The RCFPs (including DsRed-Express and DsRed-Express2) are covered by one or more of the following U.S. Patent Nos. 7,166,444; 7,157,565; 7,217,789; 7,338,784; 7,338,783; 7,537,915 6,969,597, 7,150,979 and 7,442,522.

Living Colors Fluorescent Protein Products:

Not-For-Profit Entities: Orders may be placed in the normal manner by contacting your local representative or Clontech Customer Service at 650.919.7300. At its discretion, Clontech grants Not-For-Profit Entities a non-exclusive, personal, limited license to use this product for non-commercial life science research use only. Such license specifically excludes the right to sell or otherwise transfer this product, its components or derivatives thereof to third parties. No modifications to the protein coding sequence may be made without express written permission from Clontech. Any other use of this product requires a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at [licensing@clontech.com](mailto:licensing@clontech.com).

For-Profit Entities wishing to use this product are required to obtain a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at [licensing@clontech.com](mailto:licensing@clontech.com).

Use of the Tetracycline controllable expression systems (the "Tet Technology") is covered by a series of patents including U.S. Patent Nos. 5,464,758 and 5,814,618, which are proprietary to TET Systems GmbH & Co. KG. Academic research institutions are granted an automatic license with the purchase of this product to use the Tet Technology only for internal, academic research purposes, which license specifically excludes the right to sell, or otherwise transfer, the Tet Technology or its component parts to third parties. Notwithstanding the above, academic and not-for profit research institutions whose research using the Tet Technology is sponsored by for profit organizations, which shall receive ownership to all data and results stemming from the sponsored research, shall need a commercial license agreement from TET Systems in order to use the Tet Technology. In accepting this license, all users acknowledge that the Tet Technology is experimental in nature. TET Systems GmbH & Co. KG makes no warranties, express or implied or of any kind, and hereby disclaims any warranties, representations, or guarantees of any kind as to the Tet Technology, patents, or products. All others are invited to request a license from TET Systems GmbH & Co. KG prior to purchasing these reagents or using them for any purpose. Clontech is required by its licensing agreement to submit a report of all purchasers of the Tet-controllable expression system to TET Systems. For license information, please contact: GSF/CEO, TET Systems GmbH & Co. KG, Im Neuenheimer Feld 582, 69120 Heidelberg, Germany Tel: +4962215880400, Fax: +4962215880404 eMail: [info@tetsystems.com](mailto:info@tetsystems.com) or use the electronic licensing request form via [http://www.tetsystems.com/main\\_inquiry.htm](http://www.tetsystems.com/main_inquiry.htm)

Clontech, the Clontech logo and all other trademarks are the property of Clontech Laboratories, Inc., unless noted otherwise. Clontech is a Takara Bio Company. ©2010 Clontech Laboratories, Inc.