



pTet-DualOFF Vector Map.

Description

pTet-DualOFF constitutively coexpresses the tetracycline (Tet)-controlled transcriptional activator Tet-Off[®] Advanced (1–4) and the green fluorescent protein ZsGreen1. Tet-Off 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-Off 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-Off 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-Off 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-Off 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 ColE1 origin of replication and an ampicillin resistance gene (Amp^r) to allow for propagation and selection in *E. coli*.

Use

pTet-DualOFF is used to develop stable cell lines that constitutively express Tet-Off 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).

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Location of features

- P_{CMVIE} (human cytomegalovirus immediate early promoter): 7–606
- Tet-Off Advanced: 634–1377
- IRES2 (encephalomyocarditis virus internal ribosome entry site): 1386–1970
- ZsGreen1 (*Zoanthus sp.* green fluorescent protein): 1976–2671
- SV40 polyA signal: 2709–2898
- ColE1 origin of replication: 3074–3498
- Amp^r (ampicillin resistance gene; β -lactamase): 3839–4834 (complementary)

Propagation in *E. coli*

- Recommended host strain: DH5 α TM, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 μ 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.

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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.

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