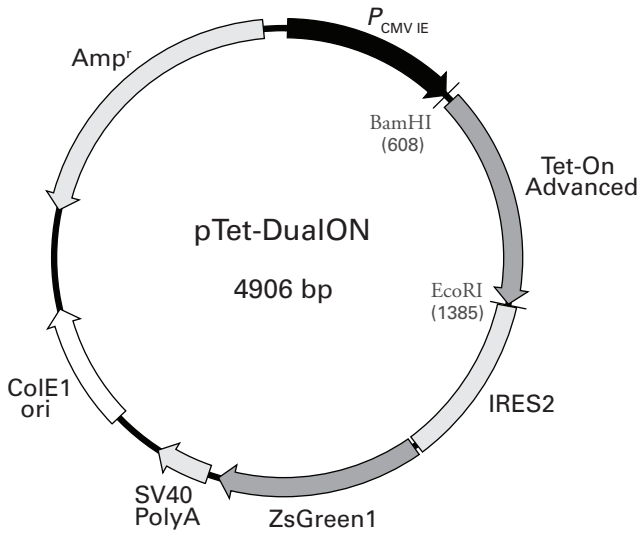
Vector Information

pTet-DualON Vector Information PT5036-5

Catalog No. 631112



pTet-DualON Vector Map.

Description

pTet-DualON constitutively coexpresses the tetracycline (Tet)-controlled transcriptional activator Tet-On® 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 ColE1 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).

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Location of features

- $P_{CMV\ IE}$ (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^r (ampicillin resistance gene; β-lactamase): 3842–4837 (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 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.