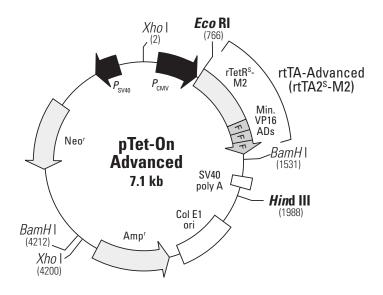
Catalog No.: 630930



Restriction Map of pTet-On-Advanced Vector. Unique restriction sites are in bold

# **Description**

pTet-On-Advanced expresses an improved version of the reverseTet (tetracycline)-controlled transactivator protein (rtTA), called rtTA-Advanced (1–4). It is more sensitive to doxycycline (Dox) and yields lower background expression than the original rtTA used in theTet-On® System (2). The rtTA-Advanced protein is a fusion of amino acids 1–207 of a mutantTet repressor (TetR) and 39 amino acids containing three minimal "F"-type transcriptional activation domains from the VP16 protein of herpes simplex virus. It is fully synthetic, lacks cryptic splice sites, and is codon-optimized for stable expression in mammalian cells.

### Use

The pTet-On-Advanced Vector is used to develop stable Tet-On Advanced cell lines, which are hosts for a Dox-induced gene expression system. Once a vector containing a gene of interest under control of a Tet-responsive element (e.g., TRE-Tight or TRE2) is transfected into a Tet-On Advanced cell line, rtTA-Advanced binds to the TRE, and activates transcription of the gene of interest in the presence of Dox in a highly dose-dependent manner. Additional information on TRE-containing vectors and protocols describing the construction of Tet-On Advanced cell lines can be found in the Tet-On Advanced Inducible Gene Expression System User Manual (PT3898-1).



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(PR083610; published 16 August 2010)

pTet-On Advanced Vector Information

### **Location of Features**

Fragment containing P<sub>CMV</sub>: 86–677

rtTA-Advanced: 775–1521

Fragment containing the SV40 poly A signal: 1544–1977

• Col E1 origin of replication: 2344–2987

Ampicillin resistance gene:

 $\beta$ -lactamase coding sequences: 3994–3134

Neomycin/kanamycin resistance gene: 6201–5407

• SV40 promoter ( $P_{SV40}$ ) controlling expression of the neomycin/kanamycin resistance gene: 6865–6522.

## Propagation in E. coli

- Suitable host strains: DH5 $\alpha^{TM}$  and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in E. coli hosts.
- E. coli replication origin: Col E1

### References

- 1. Tet-On Advanced Inducible Gene Expression System (July 2006) Clontechniques XXI(2):1-3.
- 2. Urlinger, S., et al. (2000) Proc. Natl. Acad. Sci. USA 97(14):7963-7968.
- 3. Gossen, M. & Bujard, H. (1992) Proc. Natl. Acad. Sci. USA 89(12):5547-5551.
- 4. Gossen, M., et al. (1995) Science 268(5218):1766-1769.

#### 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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