



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

### Description

pTet-On-Advanced expresses an improved version of the reverse Tet (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 the Tet-On<sup>®</sup> System (2). The rtTA-Advanced protein is a fusion of amino acids 1–207 of a mutant Tet 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).



**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

(PR083610; published 16 August 2010)

**Location of Features**

- Fragment containing  $P_{CMV}$  : 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 $\alpha$ <sup>TM</sup> and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100  $\mu$ g/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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