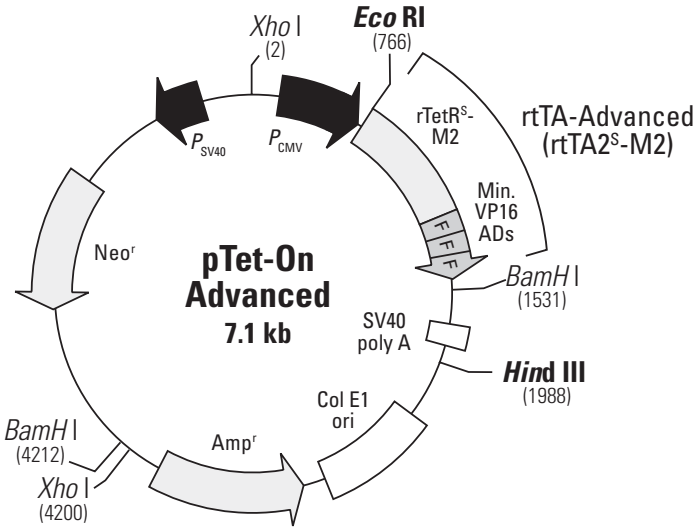
Vector Information

pTet-On Advanced Vector Information PT3899-5

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 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® 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

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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:
  β-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: DH5α 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 attached sequence file has been 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.