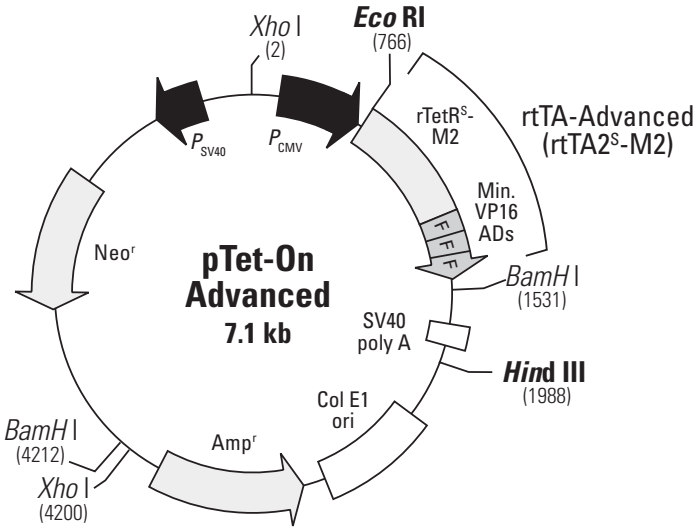
Vector Information

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).

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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 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.