Certificate of Analysis

Tet-On® 3G Vector Set (with ZsGreen1)

Table of Contents

Catalog No.	**Lot Number**
631159 (Not sold separately)	Specified on product label.

Description

The Tet-On 3G Vector Set (with ZsGreen1) is used to create tightly regulated and highly responsive tetracycline (Tet)-inducible mammalian expression systems that are turned on by the addition of doxycycline to the culture medium. The Tet-On 3G Vector Set (with ZsGreen1) allows the simultaneous expression of a gene of interest and a green fluorescent protein marker.

Package Contents

- 20 µl pCMV-Tet3G Vector (500 ng/µl)
- 20 µl pTRE3G-ZsGreen1 Vector (500 ng/µl)
- 20 µl pTRE3G-Luc Control Vector (500 ng/µl)
- 40 µl Linear Hygromycin Marker (50 ng/µl)
- 40 µl Linear Puromycin Marker (50 ng/µl)

Storage Conditions

- Store plasmids at –20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Storage Buffer

- 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)

Takara Bio USA, Inc.
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: techUS@takarabio.com

United States/Canada 800.662.2566
Asia Pacific +1.650.919.7300
Europe +33.(0)1.3904.6880
Japan +81.(0)77.565.6999

Shipping Conditions

- Dry ice (–70°C)

Product Documents

Documents for our products are available for download at **takarabio.com/manuals**
The following documents apply to this product:

- Tet-On 3G Expression Systems User Manual (PT5148-1)

pCMV-Tet3G Vector Information

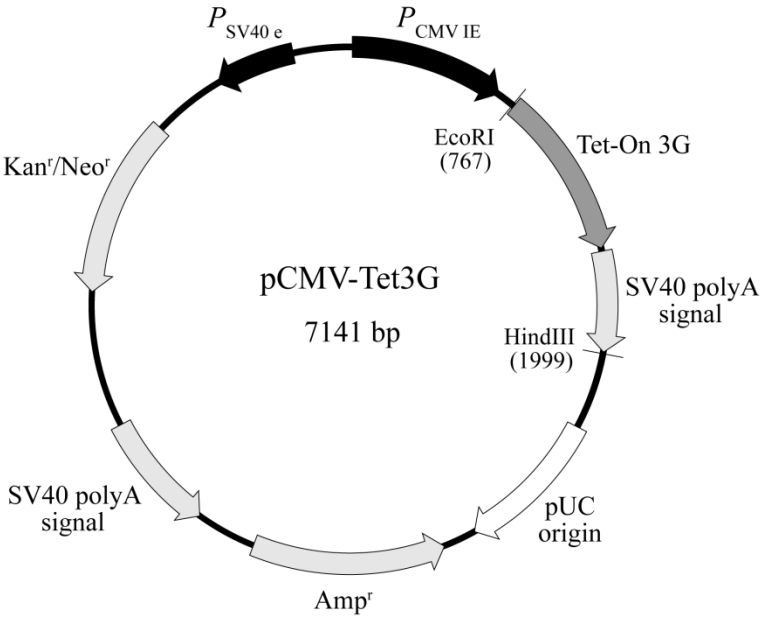

Figure 1. pCMV-Tet3G Vector Map.

Description

The pCMV-Tet3G Vector expresses Tet-On 3G, a tetracycline-controlled transactivator that exhibits high activity in the presence of the inducer doxycycline (Dox), and exceptionally low activity in its absence. Tet-On 3G results from the fusion of amino acids 1–207 of a mutant Tet repressor (TetR) to 39 amino acids that form three minimal "F"-type transcriptional activation domains from the herpes simplex virus VP16 protein. Tet-On 3G was derived from Tet-On Advanced (Zhou et al. 2006; Urlinger et al. 2000; Gossen and Bujard 1992; Gossen et al. 1995); as a result, it's fully synthetic, lacks cryptic splice sites, and is codon-optimized for stable expression in mammalian cells. Compared to both of its predecessors, however, this 3rd generation Tet-On transactivator demonstrates increased sensitivity to Dox (Zhou et al. 2006). Constitutive expression of Tet-On 3G is driven by the human cytomegalovirus immediately early promoter ($P_{CMV\ IE}$).

Location of Features in pCMV-Tet3G

- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 2–688
- Tet-On 3G (transactivator gene): 775–1521
- SV40 polyA signal: 1536–1991
- pUC origin of replication: 2342–2996
- Amp^r (ampicillin resistance gene; β-lactamase): 3144–4004 (complementary)
- SV40 polyA signal: 4275–4809 (complementary)
- Kan^r/Neo^r (kanamycin/neomycin resistance gene): 5417–6211 (complementary)
- $P_{SV40\ e}$ (SV40 early promoter): 6532–6891 (complementary)

Additional Information

pCMV-Tet3G is used to develop stable Tet-On 3G cell lines, which are hosts for Tet-inducible gene expression systems. To create a Tet-inducible expression system, a vector containing a gene of interest under the control of the Tet-inducible TRE3G promoter (P_{TRE3G}) is transfected into a Tet-On 3G cell line. The addition of Dox to the system causes Tet-On 3G to undergo a conformational change that allows it to bind to P_{TRE3G}, activating transcription of the gene of interest in a highly dose-dependent manner. Additional information on TRE-containing vectors, and protocols describing the construction of Tet-On 3G cell lines can be found in the Tet-On 3G Expression Systems User Manual (PT5148-1).

Propagation in *E. coli*

- Suitable host strain: Stellar™ Competent Cells
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

pTRE3G-ZsGreen1 Vector and pTRE3G-Luc Control Vector Information

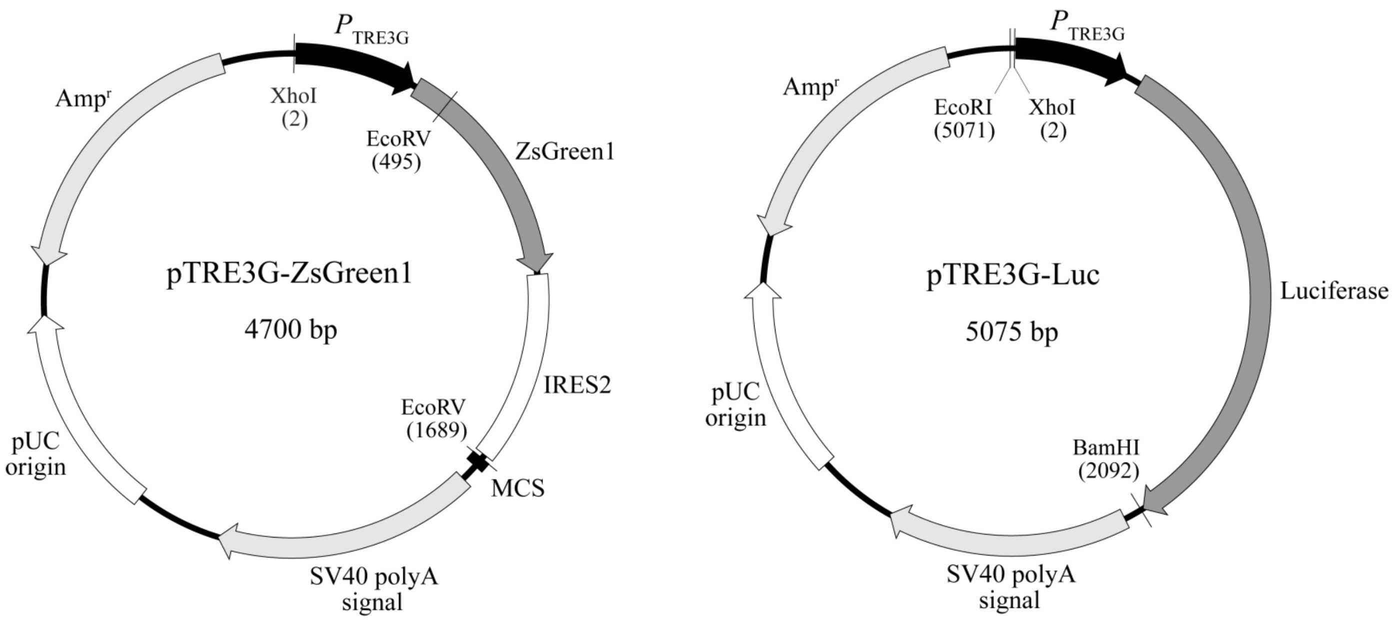

Figure 2. pTRE3G-ZsGreen1 Vector and pTRE3G-Luc Control Vector Maps.

```
       End of IRES2                            MluI                NheI      PstI      BamHI
1668   ATG GCC ACA ACC GGG CCG GAT ATC ACG CGT CAT ATG GCT AGC CTG CAG GGA TCC
       TAC CGG TGT TGG CCC GGC CTA TAG TGC GCA GTA TAC CGA TCG GAC GTC CCT AGG
```

Figure 3. pTRE3G-ZsGreen Vector Multiple Cloning Site. The internal start site (ATG) at the IRES2/MCS junction is indicated in bold.

Description

pTRE3G-ZsGreen1 is a Tet-inducible, mammalian expression vector designed to coexpress a gene of interest and the green fluorescent protein ZsGreen1 under the control of the Tet-responsive promoter P_{TRE3G}. This promoter consists of a highly optimized Tet-responsive element (TRE) just upstream of a minimal CMV promoter. P_{TRE3G} exhibits exceptionally low basal activity; it's induced by the binding of Tet-On 3G but is virtually silent in its absence. The vector is designed to be used as part of our Tet-On 3G Inducible Expression System (Cat. No. 631164).

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; Matz et al. 1999; Haas, Park, and Seed 1996). pTRE3G-ZsGreen allows Dox-inducible coexpression of ZsGreen1 and a gene of interest from a bicistronic mRNA transcript. An encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES2), positioned between ZsGreen1 and the gene of interest, facilitates cap-independent translation of the gene of interest from an internal start site at the IRES2/MCS junction (Jang et al. 1988). This ensures that a high percentage of ZsGreen1-expressing clones also express the gene of interest, allowing ZsGreen1 to be used as an indicator of inducibility and transfection efficiency, as well as a marker for selection by flow cytometry. The vector also contains a pUC origin of replication and an ampicillin resistance gene (Amp^r) to allow for propagation and selection in *E. coli*.

The pTRE3G-Luc is a Tet-inducible control vector that expresses firefly luciferase under the control of P_{TRE3G}. When used with standard luciferase detection reagents, this vector can be used as a reporter of induction efficiency (see User Manual for protocol). pTRE3G-Luc is not intended to be used as a cloning vector.

Location of Features in pTRE3G-ZsGreen1

- P_{TRE3G} (3rd generation Tet-responsive promoter): 7–382
- ZsGreen1: 389–1084
- IRES2 (encephalomyocarditis virus internal ribosome entry site): 1091–1673
- MCS (multiple cloning site): 1686–1721
- SV40 polyA signal: 1776–2573
- pUC origin of replication: 2838–3481
- Amp^r (ampicillin resistance gene; β-lactamase): 3629–4489 (complementary)

Location of Features in pTRE3G-Luc

- P_{TRE3G} (3rd generation Tet-responsive promoter): 7–382
- Luciferase: 432–2084
- SV40 polyA signal: 2151–2948
- pUC origin of replication: 3213–3856
- Amp^r (ampicillin resistance gene; β-lactamase): 4004–4864 (complementary)

Additional Information

pTRE3G-ZsGreen1 is a mammalian expression vector that allows tightly regulated, doxycycline-controlled coexpression of a gene of interest and ZsGreen1. The gene of interest must have both a start and a stop codon. The gene of interest should be cloned in-frame with the start codon at the IRES2/MCS junction (this codon is shown in bold in the MCS sequence in Figure 3, page 3; see the User Manual for details on how to use In-Fusion® to simplify your cloning). Cotransfection of pTRE3G-ZsGreen1 constructs with Linear Hygromycin or Puromycin Markers allows antibiotic selection of stable transfectants. In order to function, the system requires the presence of the Tet-On 3G transactivator protein, supplied by a stable Tet-On 3G cell line created with our Tet-On 3G Inducible Expression System (Cat. No. 631164).

Propagation in *E. coli*

- Suitable host strain: Stellar™ Competent Cells
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

Excitation and Emission of pTRE3G-ZsGreen1

- Excitation: 493 nm
- Emission: 505 nm

References

- Gossen, M. *et al.* Transcriptional activation by tetracyclines in mammalian cells. *Science* **268,** 1766–9 (1995).
- Gossen, M. & Bujard, H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl. Acad. Sci. U. S. A.* **89,** 5547–51 (1992).
- Haas, J., Park, E. C. & Seed, B. Codon usage limitation in the expression of HIV-1 envelope glycoprotein. *Curr. Biol.* **6,** 315–24 (1996).
- Jang, S. K. *et al.* A segment of the 5' nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. *J. Virol.* **62,** 2636–43 (1988).
- Matz, M. V *et al.* Fluorescent proteins from nonbioluminescent Anthozoa species. *Nat. Biotechnol.* **17,** 969–73 (1999).
- Urlinger, S. *et al.* Exploring the sequence space for tetracycline-dependent transcriptional activators: novel mutations yield expanded range and sensitivity. *Proc. Natl. Acad. Sci. U. S. A.* **97,** 7963–8 (2000).
- Zhou, X., Vink, M., Klaver, B., Berkhout, B. & Das, A. T. Optimization of the Tet-On system for regulated gene expression through viral evolution. *Gene Ther.* **13,** 1382–90 (2006).

Quality Control Data

Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Vector	Enzyme(s)	Fragment(s)
pCMV-Tet3G	EcoRI	7.1 kb
	EcoRI & HindIII	1.2 & 5.9 kb
pTRE3G-ZsGreen1	XhoI	4.7 kb
	EcoRV	1.2 & 3.5 kb
pTRE3G-Luc	XhoI	5.1 kb
	EcoRI & BamHI	2.1 & 3.0 kb
Linear Hygromycin Marker	HindIII & XbaI	0.5, 0.6 & 1.1 kb
Linear Puromycin Marker	HindIII & XbaI	0.45, 0.6, & 0.75 kb

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280}: 1.8–2.0

Functional Testing of Linear Markers

- HEK 293 cells were transfected with 200 ng of either the Linear Hygromycin Marker or the Linear Puromycin Marker. After 5 hr at 37°C, the transfection solution was removed, and the cells were given fresh medium. 48 hr later, the cells were plated in two 10 cm plates. 48 hr after plating, medium containing either hygromycin or puromycin (depending on the linear marker used to transfect the cells) was added to the plates. After 2–3 weeks, >20 clones were identified.

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

Notice to Purchaser

Tet-On® 3G Vector Set (with ZsGreen1)

CATALOG NO.

631159

NOTICE TO PURCHASER:

Our products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, in vitro diagnostic purposes, therapeutics, or in humans. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without prior written approval of Takara Bio USA, Inc.

Your use of this product is also subject to compliance with the licensing requirements listed below and described on the product´s web page at http://www.takarabio.com. It is your responsibility to review, understand and adhere to any restrictions imposed by these statements.

STATEMENT 24

The RCFPs (including DsRedExpress, DsRedExpress2, and E2-Crimson) are covered by one or more of the following U.S. Patent Nos. 7,166,444; 7,157,565; 7,217,789; 7,338,784; 7,338,783; 7,537,915; 6,969,597; 7,150,979; 7,442,522 and 8,012,682.

STATEMENT 72

Living Colors Fluorescent Protein Products: Not-For-Profit Entities: Orders may be placed in the normal manner by contacting your local representative or Takara Bio USA, Inc. Customer Service. Any and all uses of this product will be subject to the terms and conditions of the Non-Commercial Use License Agreement (the “Non-Commercial License”), a copy of which can be found below. As a condition of sale of this product to you, and prior to using this product, you must agree to the terms and conditions of the Non-Commercial License. Under the Non-Commercial License, Takara Bio USA, Inc. grants Not-For-Profit Entities a non-exclusive, non-transferable, non-sublicensable and limited license to use this product for internal, non-commercial scientific research use only. Such license specifically excludes the right to sell or otherwise transfer this product, its components or derivatives thereof to third parties. No modifications to the product may be made without express written permission from Takara Bio USA, Inc. Any other use of this product requires a different license from Takara Bio USA, Inc. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@takarabio.com. For-Profit Entities wishing to use this product are required to obtain a license from Takara Bio USA, Inc. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@takarabio.com.

STATEMENT 42

Use of the Tetracycline controllable expression systems (the "Tet Technology") is covered by a series of patents including U.S. Patent # 7541446, # 8383364, # 9181556 , European patents EP # 1200607, # 1954811, #2352833

Takara Bio USA, Inc.
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: techUS@takarabio.com

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.565.6999

4/27/2018

and corresponding patent claims outside these regions which are proprietary to TET Systems GmbH & Co. KG. Academic research institutions are granted an automatic license with the purchase of this product to use the Tet Technology only for internal, academic research purposes, which license specifically excludes the right to sell, or otherwise transfer, the Tet Technology or its component parts to third parties. Notwithstanding the above, academic and not-for profit research institutions whose research using the Tet Technology is sponsored by for profit organizations, which shall receive ownership to any data and results stemming from the sponsored research, shall need a commercial license agreement from TET Systems in order to use the Tet Technology. In accepting this license, all users acknowledge that the Tet Technology is experimental in nature. TET Systems GmbH & Co. KG makes no warranties, express or implied or of any kind, and hereby disclaims any warranties, representations, or guarantees of any kind as to the Tet Technology, patents, or products. All others are invited to request a license from TET Systems GmbH & Co. KG prior to purchasing these reagents or using them for any purpose. Takara Bio USA, Inc. is required by its licensing agreement to submit a report of all purchasers of the Tet-controllable expression system to TET Systems.

For license information, please contact:
GSF/CEO
TET Systems GmbH & Co. KG,
Im Neuenheimer Feld 582
69120 Heidelberg
Germany
Tel: +49 6221 5880400
Fax: +49 6221 5880404
email: info@tetsystems.com
or use the electronic licensing request form via http://www.tetsystems.com/ip-licensing/licensing/for-profit-research

TRADEMARKS: