Certificate of Analysis

pCMV-Tet3G Vector

Catalog No.	Amount	Lot Number
631335 (Not sold separately) Sold as part of 631159, 631164, 631165, 631166, 631168, 631337, 631338, 631339, 635079, 635084	20 µl (500 ng/µl)	Specified on product label.

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 (1–4); 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, this 3rd generation Tet-On transactivator demonstrates increased sensitivity to Dox (1). Constitutive expression of Tet-On 3G is driven by the human cytomegalovirus immediately early promoter (PCMV IE). Note: An EF1α version of this vector is available for cell lines in which the CMV promoter is silenced.

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 (PTRE3G) 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 PTRE3G, activating transcription of the gene of interest in a highly dose-dependent manner.

Package Contents

- 20 µl pCMV-Tet3G Vector

Storage Conditions

- Store plasmid 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-HCI (pH 8.0)
- 1 mM EDTA (pH 8.0)

Concentration

- 500 ng/µl

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(050918)

Certificate of Analysis

Cat. No. 631335

pCMV-Tet3G Vector (Not sold separately)

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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)

Propagation in *E. coli*

- Recommended host strain: Stellar™ Competent Cells (Cat. No. 636763).
- Selectable marker: plasmids confer resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

References

1. Zhou, X. *et al*. (2006) *Gene Ther*. **13**(19):1382-1390.
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.

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)	Size (kb)
pCMV-Tet3G	EcoRI	7.1 kb
	EcoRI & HindIII	1.2 & 5.9 kb

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

Notice to Purchaser

pCMV-Tet3G Vector

CATALOG NO.

631335

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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 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.

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5/9/2018