Certificate of Analysis

pTRE3G-ZsGreen1 Vector Set

Catalog No.	Amount	Lot Number
631176 (Not sold separately) Sold as part of 631164, 631172 & 631348	Each	Specified on product label.

Description

pTRE3G-ZsGreen1 Vector Set provides an inducible mammalian expression vector that is tightly regulated, and highly responsive to Tet-On®, Tet-Off®, and Tet-Express™ transactivators (Gossen and Bujard 1992). The simultaneous expression of a gene of interest and a green fluorescent protein marker is driven from the inducible P_{TRE3G} promoter, which produces 5–20-fold less background expression than the P_{Tight} promoter. The vector set also includes: a control vector that expresses luciferase in response to transactivation; and two linear selection markers for hygromycin and puromycin resistance.

Package Contents

- 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.

Expiration Date

- Specified on product label.

Storage Buffer

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

Concentration

- pTRE3G-ZsGreen1 Vector and pTRE3G-Luc Control Vector: 500 ng/µl
- Linear Hygromycin Marker and Linear Puromycin Marker: 50 ng/µl

Shipping Conditions

- Dry ice

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(042423)

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
- Tet-Express Inducible Expression Systems User Manual
- pTRE3G-ZsGreen1 Vector Information
- pTRE3G-ZsGreen1 Vector Sequence in GenBank Format
- pTRE3G-Luc Control Vector Information
- pTRE3G-Luc Control Vector Sequence in GenBank Format

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: high-copy-number ColE1/pMB1/pBR322/pUC origin of replication

Excitation and Emission Maxima of mCherry

- Excitation: 493 nm
- Emission: 505 nm

References

Gossen, M. & Bujard, H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl. Acad. Sci.* **89,** 5547–5551 (1992).

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	Enzymes	Fragments
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

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

Linear Selection Marker Identity

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

Marker	Enzymes	Fragments
Linear Hygromycin Marker	HindIII & XbaI	0.5, 0.6 & 1.1 kb
Linear Puromycin Marker	HindIII & XbaI	0.45, 0.6, & 0.75 kb

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 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

pTRE3G-ZsGreen1 Vector Set

CATALOG NO.

631176

NOTICE TO PURCHASER:

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. 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 our prior written approval.

Your use of this product is also subject to compliance with the licensing requirements, listed below if applicable, 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 42

Use of the Tetracycline controllable expression systems (the "Tet Technology") is covered by a series of patents including U.S. Patent # 8383364, # 9181556 , European patents EP # 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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5/22/2025