# Certificate of Analysis



## pTRE3G Vector Set

Catalog No.

631173 (Not sold separately) Sold as a part of 631167 & 631168 **Amount** Each

**Lot Number** 

Specified on product label.

## **Description**

The pTRE3G Vector Set provides an inducible mammalian expression vector that is tightly regulated, and highly responsive to Tet-On®, Tet-Off®, and Tet-Express<sup>TM</sup> transactivators (Gossen, M. & Bujard 1992). Expression of a protein of interest is driven from the inducible  $P_{\text{TRE3G}}$  promoter, which produces 5–20-fold less background expression than the  $P_{\text{Tight}}$  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 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 Vector and pTRE3G-Luc Control Vector: 500 ng/μl
- Linear Markers: 50 ng/μl

### **Shipping Conditions**

Dry ice

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### **Product Documents**

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

- Tet-On 3G Inducible Expression Systems User Manual
- Xfect Transfection Reagent Protocol-At-A-Glance
- pTRE3G Vector Information
- pTRE3G-Luc Control Vector Information

### Propagation in *E. coli*

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

#### References

1. Gossen, M. & Bujard, H. (1992) Proc. Natl. Acad. Sci. USA 89(12):5547-5551.

## **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	Size
pTRE3G	XhoI	3.4 kb
	XbaI	0.9 & 2.6 kb
pTRE3G-Luc	XhoI	5.1 kb
_	EcoRI & BamHI	2.1 & 3.0 kb

- Vector identity was confirmed by sequencing.
- A<sub>260</sub>/A<sub>280</sub>: 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	Enzyme	Size
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.

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631173

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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 # 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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2/24/2023