Certificate of Analysis

pmRi-ZsGreen1 Vector

Catalog No.	Amount	Lot Number
631121	20 µg	Specified on product label.

Description

The pmRi-ZsGreen1 Vector is a Tet-inducible mammalian expression vector that allows the coexpression of a user-generated microRNA (miRNA) and a green fluorescent protein, ZsGreen1, to be controlled by a Tet System transactivator and doxycycline (Gossen and Bujard 1992; Urlinger et al. 2000). The TRE-based promoter, P_{Tight}, regulates the expression of the ZsGreen1 mRNA transcript, which contains your miRNA precursor sequence embedded in its 3' UTR. Inducibility requires that a Tet System transactivator (e.g., Tet-On® Advanced) also be expressed in the target cells. To select stable cell lines, the pmRi-ZsGreen1 Vector must be cotransfected with one of the provided linear selection markers.

Package Contents

- 20 µg pmRi-ZsGreen1 Vector (500 ng/µl)
- 20 µg pTRE-Tight-Luc Vector (500 ng/µl)
- 40 µl Linear Hygromycin Marker (50 ng/µl)
- 40 µl Linear Puromycin Marker (50 ng/µl)

Storage Conditions

- Store all components at –20°C.
- Spin tubes 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, 1 mM EDTA (pH 8.0)

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:

- pmRi-ZsGreen1 Vector Information
- Mir-X Inducible miRNA Systems User Manual

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(041018)

References

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).

Urlinger, S. *et al.* Exploring the sequence space for tetracycline-dependent transcriptional activators: Novel mutations yield expanded range and sensitivity. *Proc. Natl. Acad. Sci.* **97,** 7963–7968 (2000).

Quality Control Data

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

Vector	Enzyme(s)	Fragment(s)
pmRi-ZsGreen1 Vector	BamHI	3.3 kb
	EcoRI/BamHI	2.6 & 0.7 kb
pTRE-Tight-Luc Vector	BamHI/NheI	2.6 & 1.6 kb
	XbaI	4.2 kb
Linear Hygromycin Marker	HindIII/XbaI	1.05, 0.6 & 0.45
Linear Puromycin Marker	HindIII/XbaI	0.75, 0.6 & 0.45

   - A_{260}/A_{280}: 1.8–2.0
   - Vector identities were confirmed by sequencing

2. Functional Testing of Linear Markers
   As a functional test, HEK 293 cells were transfected with 200 ng of Linear Hygromycin or Puromycin Marker. After 5 hr at 37°C, the transfection solution was removed, and cells were given fresh media. 48 hr later, cells were plated in two 10 cm plates. 48 hr after plating, media containing hygromycin or puromycin was added to the plates. After 2–3 weeks, totals of >20 clones were identified for each marker.

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

Notice to Purchaser

pmRi-ZsGreen1 Vector

CATALOG NO.

631121

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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.

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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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