Certificate of Analysis

pmRi-mCherry Vector

Catalog No.	Amount	Lot Number
631119	20 µg	Specified on product label.

Description

The pmRi-mCherry Vector is a Tet-inducible mammalian expression vector that allows the coexpression of a user-generated microRNA (miRNA) and a red fluorescent protein, mCherry, 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 mCherry 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-mCherry Vector must be cotransfected with one of the provided linear selection markers.

Package Contents

- 20 µg pmRi-mCherry 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-mCherry 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-mCherry 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-mCherry Vector

CATALOG NO.

631119

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STATEMENT 44

The DsRed-Monomer and the Fruit Fluorescent Proteins are covered by one or more of the following U.S. Patents: 7,005,511; 7,157,566; 7,393,923 and 7,250,298.

STATEMENT 72

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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.

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4/10/2018

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