Certificate of Analysis

Tet-On® Advanced IRES Fluorescent Vector Set

Catalog No.	Amount	Lot Number
631112	20 µg	Specified on product label.

Description

The Tet-On Advanced IRES Fluorescent Vector Set allows you to establish a tightly regulated and highly responsive inducible system for simultaneous coexpression of your gene of interest and a fluorescent protein marker. The system is turned on by adding doxycycline to the culture medium. The pTet-DualON Vector expresses the Tet-ON Advanced transactivator along with the green fluorescent protein, ZsGreen1, to allow you to sort for high-expression of the transactivator in target cells. With pTRE-Dual2, Tet-inducible coexpression of your gene of interest and the red fluorescent protein, mCherry, is controlled by the TRE-based promoter, P_{Tight}. Coexpression of two genes from a single transcription unit in each of these vectors is made possible by an internal ribosome entry site (IRES2) that separates the two coding sequences. pTRE-Dual2-Luc is an inducible control vector that coexpresses mCherry and luciferase. To select stable cell lines, the vectors must be cotransfected with one of the provided linear selection markers.

Package Contents

- 20 µg pTet-DualON Vector (500 ng/µl)
- 20 µg pTRE-Dual2 Vector (500 ng/µl)
- 20 µg pTRE-Dual2-Luc 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.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Storage Buffer

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

Shipping Conditions

- Dry ice (–70°C)

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(050818)

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Product Documents

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

- Tet-On Advanced Inducible Gene Expression Systems User Manual (PT3898-1)
- pTRE-DualON Vector Information (PT5036-5)
- pTRE-Dual2 Vector Information (PT5038-5)
- pTRE-Dual2-Luc Vector Information (PT5039-5)

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)	Fragment(s)
pTet-DualON Vector	BamHI	4.9 kb
	BamHI, EcoRI	4.1 & 0.8 kb
pTRE-Dual2 Vector	EcoRI	3.9 kb
	EcoRI, BamHI	2.6 & 1.3 kb
pTRE-Dual2-Luc Control Vector	EcoRI	5.6 kb
	EcoRI, BamHI	4.3 & 1.3 kb
Linear Hygromycin Marker	HindIII, XbaI	1.1, 0.6 & 0.5 kb
Linear Puromycin Marker	HindIII, XbaI	0.8, 0.6 & 0.5 kb

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

Functional Testing of Linear Markers

As a functional test, HEK 293 cells were transfected with 200 ng of Linear Hygromycin or Puromycin Marker using a liposome-mediated transfection method. 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

Tet-On® Advanced IRES Fluorescent Vector Set

CATALOG NO.

631112

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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 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

Living Colors Fluorescent Protein Products:

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4/6/2021

Notice to Purchaser

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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STATEMENT 24

DsRed2, DsRedExpress, DsRedExpress2, E2-Crimson, HcRed and pTimer are covered by one or more of the following U.S. Patent Nos. 7,166,444; 7,150,979; 7,157,565; 7,183,399; 7,858,844; 8,093,450; 8,461,769 and non-U.S. international equivalents in some countries.

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