Certificate of Analysis

In the interest of conserving resources, we are no longer shipping manuals with products. Please visit **www.clontech.com/manuals** to obtain an electronic version.

PRODUCT: pTRE-Cycle2 Vector

CATALOG NO.
631116

AMOUNT
20 µg

LOT NUMBER
Specified on product label.

STORAGE BUFFER
10 mM Tris-HCl (pH 8.0)
1 mM EDTA (pH 8.0)

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.

SHIPPING CONDITIONS
Dry ice (–70°C)

PRODUCT USER MANUALS
User manuals for Clontech® products are available for download at **www.clontech.com/manuals**.
- Tet-On Advanced Inducible Gene Expression Systems User Manual (PT3898-1)
- Tet-Off Advanced Inducible Gene Expression Systems User Manual (PT3945-1)

DESCRIPTION
pTRE-Cycle2 is a bidirectional, Tet-inducible expression vector that allows you to reversibly regulate (i.e., cycle) the amount of a protein of interest in mammalian cells while coexpressing the red fluorescent protein mCherry. Expression of both proteins is tightly regulated by the bidirectional, TRE-based promoter, $P_{Tight\text{-}Bi}$. Expression of the gene of interest is controlled by an additional mechanism, since the resulting protein is fused to an N-terminal ProteoTuner™ destabilization domain (DD), which causes rapid proteasomal degradation of any protein to which it is fused. The degradation process can be reversed repeatedly by adding the stabilizing ligand Shield1 and then removing it from the culture medium. mCherry is coexpressed with the tagged protein, but does not contain a DD-tag, and thus is subject only to Tet-based regulation. The vector is intended for use with any Tet-On® or Tet-Off® Advanced Expression System. To select stable cell lines, the vector must be cotransfected with one of the provided linear selection markers.

PACKAGE CONTENTS
- 20 µg pTRE-Cycle2 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)

OTHER
- pTRE-Cycle2 Vector Information (PT5046-5)

FOR RESEARCH USE ONLY

QUALITY CONTROL DATA

See back of page.

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(PA013557)

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)
pTRE-Cycle2 Vector	BamHI	3.9 kb
	XhoI/BamHI	3.2 & 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 also 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.

Notice to Purchaser

pTRE-Cycle2 Vector

CATALOG NO.

631116

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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 Nos. 6087166, 6271341, 7541446, 8383364, European Patents: EP 0990030, 1954811, 2050818, 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 all 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. Clontech is required by its licensing agreement to submit a report of all purchasers of the Tet-controllable expression system to TET Systems. For license information, please contact: GSF/CEO TET Systems GmbH & Co. KG, Im Neuenheimer Feld 582 69120 Heidelberg Germany Tel: +49 6221 5880400 Fax: +49 6221 5880404 email: info@tetsystems.com or use the electronic licensing request form via www.tetsystems.com/main_inquiry.htm

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 57

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10/13/2015

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Notice to Purchaser

This product is covered by U.S. Patent No. 8,173,792.

STATEMENT 72

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TRADEMARKS:

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