Certificate of Analysis

pTRE-Cycle1 Vector

Catalog No.	Amount	Lot Number
631115	20 µg	Specified on product label.

Description

pTRE-Cycle1 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 a second protein that is not cycled. Expression of both proteins is tightly regulated by the bidirectional, TREbased promoter, $P_{Tight\text{-}Bi}$. Expression of the first 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. The second gene of interest, which is cloned into a separate multiple cloning site, will be coexpressed with the tagged protein, but will not acquire 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-Cycle1 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-HCI (pH 8.0)
- 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:

Takara Bio USA, Inc.
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: techUS@takarabio.com

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.565.6999
(050118)			

- Tet-On Advanced Inducible Gene Expression Systems User Manual (PT3898-1)
- Tet-Off Advanced Inducible Gene Expression Systems User Manual (PT3945-1)
- pTRE-Cycle1 Vector Information (PT5045-5)

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)	Size (kb)
pTRE-Cycle1 Vector	BamHI	3.2 kb
	XhoI/BamHI	2.5, 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 kb
Linear Puromycin Marker	HindIII/XbaI	0.75, 0.6 & 0.45 kb

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280}: 1.8–2.0

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

pTRE-Cycle1 Vector

CATALOG NO.

631115

NOTICE TO PURCHASER:

Our products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, in vitro diagnostic purposes, therapeutics, or in humans. 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 prior written approval of Takara Bio USA, Inc.

Your use of this product is also subject to compliance with the licensing requirements listed below and described on the product´s web page at http://www.takarabio.com. It is your responsibility to review, understand and adhere to any restrictions imposed by these statements.

STATEMENT 57

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

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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TRADEMARKS:

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