InDOXible™ Tet-Activated Lentiviral cDNA Expression System

CELLECTA

Discovery is yours™

Convenient Doxycycline-Inducible cDNA Expression

- Ultra-low background minimizes leakiness in polyclonal cultures so clonal selection is unnecessary
- Optional fluorescent sensor enables real-time monitoring of induction and repression
- Easy-to-use, complete, all-in-one and two-vector inducible lentiviral expression systems

Cellecta **InDOXible™ Tet-Activated cDNA Lentiviral Expression System** enables convenient doxycycline-inducible cDNA expression.

Available as an all-in-one lentiviral vector system and as a two-vector inducible system

(1) **One-Vector Tet-Activated System:** The transactivator that regulates gene induction is on the same construct as the inducible gene of interest. Transduction of the single lentiviral construct provides all the elements needed to tightly regulate inducible cDNA expression.

(2) **Two-Vector System:** The inducible cDNA that is under the control of the responsive promoter is on a vector without the transactivator. The transactivator is on its own vector and transduced separately into cells, and so can be used to create a panel of transactivator cells set up for introduction of different genes of interest.

How Does It Work?

Fusion of a bacterial tet-repressor protein with the VP16 activating domain creates a transactivator that binds, in a tetracycline-dependent manner, the tet-responsive element (TRE) derived from bacterial promoters. Cellecta has optimized the transactivator to ensure very tightly regulated activation of the minimal CMV promoter driving expression of the cDNA of interest. As a result, selection of low background clones is typically unnecessary. Background levels of antibiotic-selected, transduced polyclonal cell cultures is minimal.

The two variations of the tetracycline activator (tTA and rtTA) enable activation with either the addition or removal

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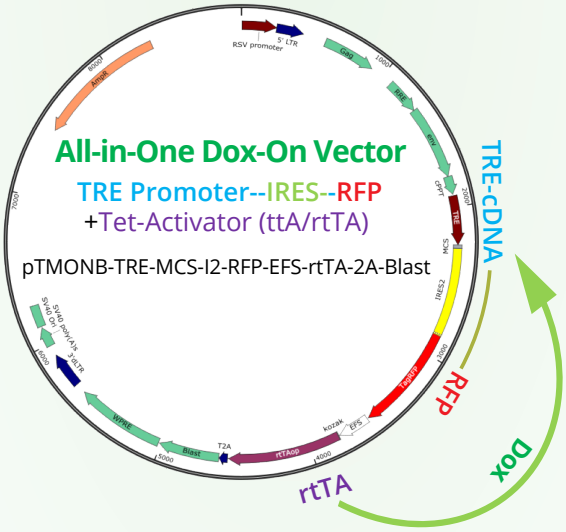
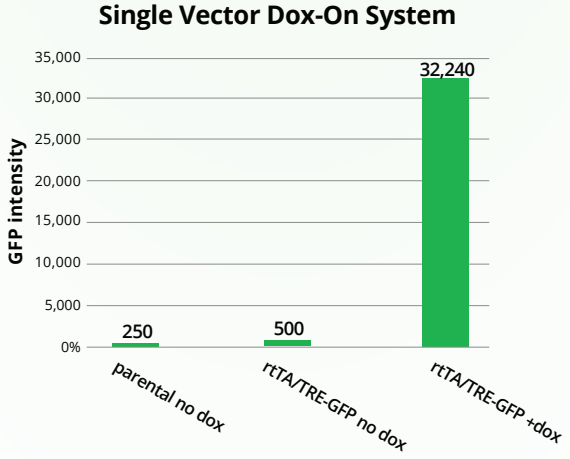
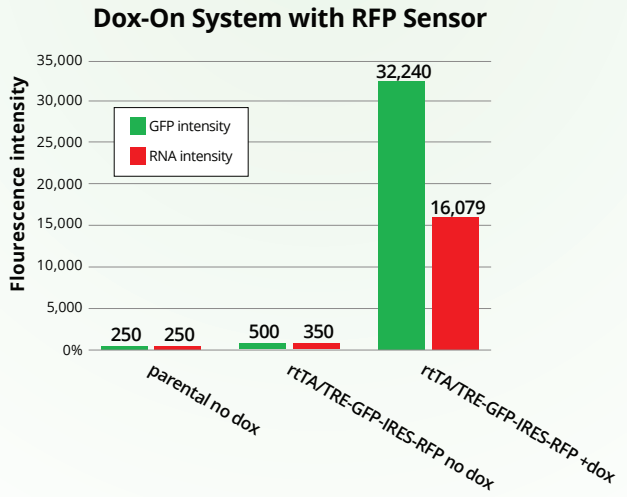


All-in-One Lentiviral Dox-On and Dox-Off Constructs (as shown) and Two-Vector TRE-Lentiviral Vector with separate Dox-On and Dox-Off Activator constructs (not shown), are available. With the Sensor option, the RFP Sensor is expressed on the same transcript as the cDNA of interest but separated by an IRES (internal ribosome entry site). As a result, it is co-expressed with the cDNA of interest.



U2OS cells were transduced with **all-in-one rtTA/TRE-GFP** (blastR) reporter vector at MOI <1, then selected with blasticidin for 1 week. Selected cells were cultured for 2 additional days with or without doxycycline, and assayed for GFP fluorescence intensity by flow cytometry. Data shows expression level of the GFP protein with and without dox.

Note: These are polyclonal cultures. There was no clonal selection. There is very little leakiness.



U2OS cells were transduced at MOI <1 with the **TRE-GFP-IRES-RFP reporter vector**, selected with blasticidin for 1 week, and cultured for 2 additional days with or without doxycycline. Detection levels for both GFP and RFP fluorescence intensity was assessed by flow cytometry. Activation of the RFP sensor signal correlates strongly with the increased activation of the GFP cDNA of interest.

of the tetracycline analog doxocycline (dox). Since all the elements are provided on lentiviral vectors, they can be easily and effectively introduced into any mammalian cell line.

Fluorescent Sensor for Real-Time Induction Monitoring

A fluorescent reporter (RFP), linked by an internal ribosome entry site (IRES) to the cDNA transcript, is available with both the single-vector and dual-vector InDOXible Tet-Activated vectors. With this IRES configuration, cell fluorescence increases or decreases in parallel with the cloned cDNA, allowing monitoring of induction. This fluorescent sensor feature allows convenient confirmation of gene modulation during experiments.

Ordering Information

Catalog #	Description	Quantity
SVTTAH-P	tTA (Dox-Off) Expression Vector with Hygro (pTTAH-SFFV-tTA-UBC-Hygro)	25 ug
SVTTAP-P	tTA (Dox-Off) Expression Vector with Puro (pTTAP-SFFV-tTA-UBC-Puro)	25 ug
SVRTTAH-P	rtTA (Dox-On) Expression Vector with Hygro (pRTTAH-SFFV-rtTA-UBC-Hygro)	25 ug
SVRTTAP-P	rtTA (Dox-On) Expression Vector with Puro (pRTTAP-SFFV-rtTA-UBC-Puro)	25 ug
SVTTAH-V	tTA (Dox-Off) Expression Vector with Hygro (pTTAH-SFFV-tTA-UBC-Hygro, virus)	1 x 10^6 TU
SVTTAP-V	tTA (Dox-Off) Expression Vector with Blast (pTTAP-SFFV-tTA-UBC-Puro, virus)	1 x 10^6 TU
SVRTTAH-V	rtTA (Dox-On) Expression Vector with Hygro (pRTTAH-SFFV-rtTA-UBC-Hygro, virus)	1 x 10^6 TU
SVRTTAP-V	rtTA (Dox-On) Expression Vector with Hygro (pRTTAP-SFFV-rtTA-UBC-Puro, virus)	1 x 10^6 TU
SVTUB-P	Two-Vector Dox-Responsive TRE Cloning Vector (pTMUB-TRE-MCS-UBC-Blast)	25 ug
SVTURB-P	Two-Vector Dox-Responsive TRE Cloning Vector w/Sensor (pTMURB-TRE-MCS-I2-RFP-UBC-Blast)	25 ug
SVTTATB-P	All-in-One Dox-Off Cloning Vector (pTMOFFB-TRE-MCS-EFS-tTA-2A-Blast)	25 ug
SVTTATRB-P	All-in-One Dox-Off w/Sensor Cloning Vector (pTMOFFRB-TRE-MCS-I2-RFP-EFS-tTA-2A-Blast)	25 ug
SVRTTATB-P	All-in-One Dox-On Cloning Vector (pTMONB-TRE-MCS-EFS-rtTA-2A-Blast)	25 ug
SVRTTATRB-P	All-in-One Dox-On w/Sensor Cloning Vector (pTMONRB-TRE-MCS-I2-RFP-EFS-rtTA-2A-Blast)	25 ug

For more information on these and other Cellecta products, visit www.cellecta.com or email us at info@cellecta.com