Mammalian Expression Systems

Tet-Express™—Fast, Inducible Expression

The quickest, simplest Tet-inducible expression system yet

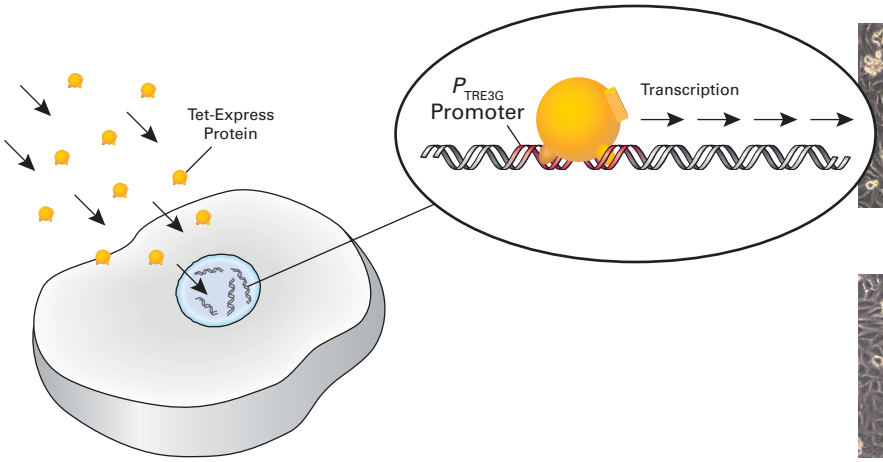
- **Fast to set up—no need to create a Tet-On® or Tet-Off® cell line**
- **Fast to induce—reach 80% of maximal expression in just 2 hours**
- **Doxycycline-free protocol—instead just add Tet-Express transactivator directly to cells**

Clontech's **Tet-Express Inducible Expression Systems** are a faster, simpler adaptation of our powerful, tightly-regulated Tet-On/Tet-Off expression systems (1–3). Unlike Tet-On/Tet-Off, the Tet-Express system requires only a single vector, the Tet-Express transducible protein, and a doxycycline-free protocol. To induce expression, simply apply a few microliters of Tet-Express to the culture medium of cells in which your gene is under the control of any TRE promoter. Tet-Express makes tetracycline-controlled transcription a rapid process for all cell types, and is particularly advantageous for cell types that are not amenable to sequential rounds of clonal selection.

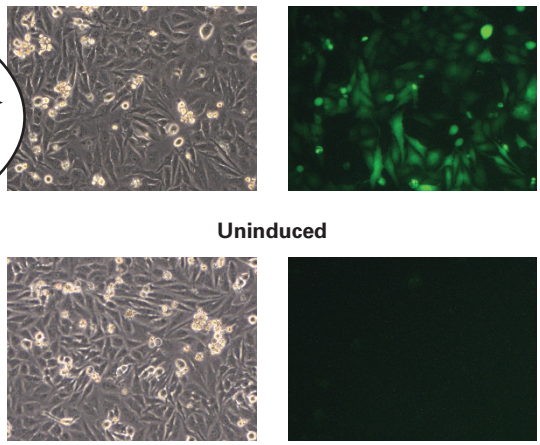
What is Tet-Express?

Tet-Express is a version of Clontech's Tet-Off Advanced transcriptional activator (transactivator) protein that has been modified and optimized for self-transduction, i.e., it has the ability to transport itself across cell membranes into the nucleus via protein transduction pathways. Since Tet-Express binds and activates expression in the absence of tetracyclines, doxycycline is not required for gene activation.

An Intensifier Reagent is included with each vial of Tet-Express. We have found that this reagent increases protein transduction efficiency.



Tet-Express Induction of AcGFP1



Uninduced

The Tet-Express System, like Clontech's Tet-On 3G System, expresses your transgene from a vector containing the tightly-regulated inducible promoter P_{TRE3G}. However, unlike the Tet-On 3G system, you do not need to create a double-stable cell line that expresses the tetracycline transactivator, since the self-transducing Tet-Express transactivator protein is added directly to your cells.

Clontech Laboratories, Inc.
A Takara Bio Company
www.clontech.com
United States/Canada: +1.800.662.2566 • Asia Pacific: +1.650.919.7300 • Europe: +33.(0)1.3904.6880 • Japan: +81.(0)77.543.6116

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Rapid Induction of Gene Expression

Gene expression with and without Tet-Express treatment was compared in HeLa cells that contain a stably integrated luciferase gene under the control of a TRE promoter. In the presence of Tet-Express, luciferase expression increased rapidly, to over 80% of maximum after 2 hours, while no expression took place in the absence of Tet-Express treatment (Figure 1).

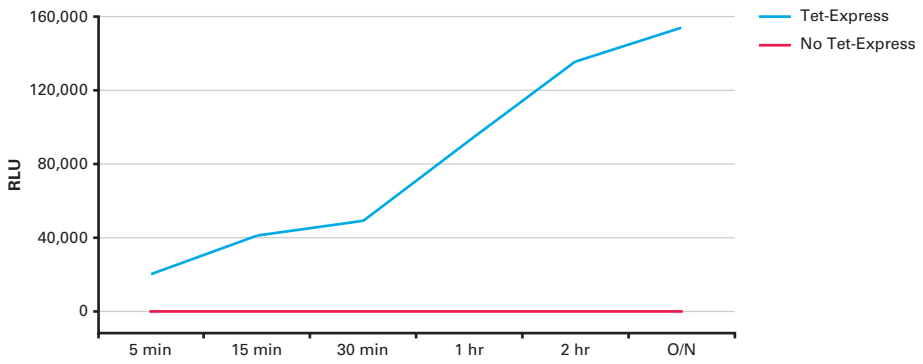

Figure 1. Tet-Express rapidly induces gene expression from TRE promoters. HeLa cells containing a stably integrated luciferase expression cassette under the control of a TRE promoter were treated with Tet-Express and compared to untreated cells from the same HeLa cell culture at different time points. 88% of maximal expression was achieved in just 2 hr.

High Induction, Low Background in a wide variety of Cell Types

Since the Tet-Express system makes it unnecessary to create a separate cell line expressing the transactivator, it eliminates the need for multiple rounds of clonal selection, making it especially useful for cell types that are not amenable to sequential rounds of clonal selection, such as primary cells. Tet-Express has been shown to be very effective across a broad range of cell lines (Figure 2).

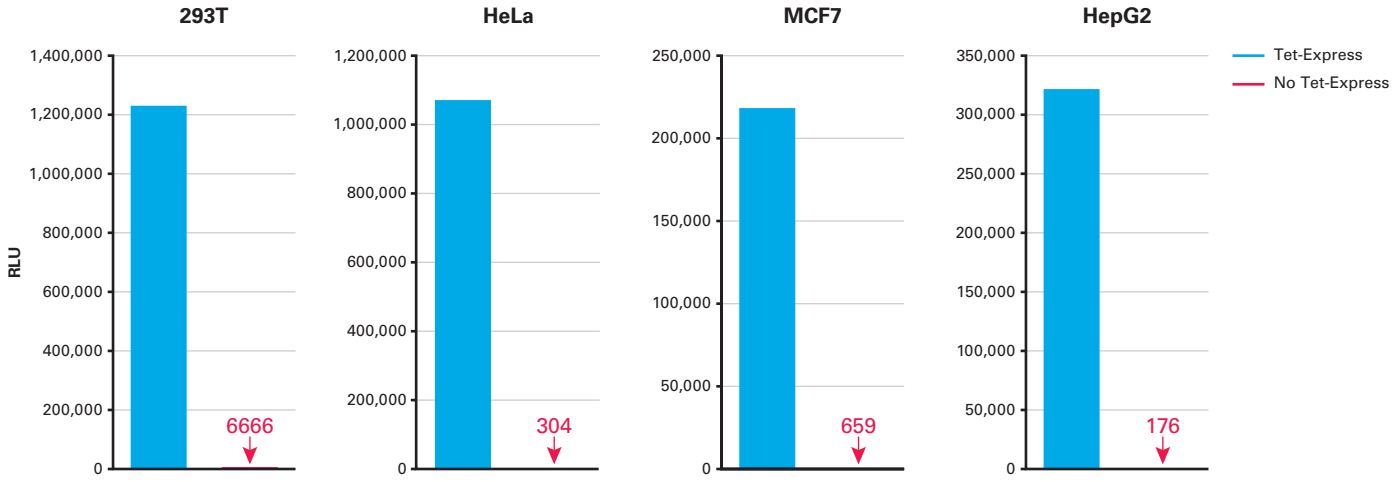

Figure 2. Tet-Express works well with a broad range of cell types. Cultures of 293T cells, HeLa cells, MCF7 cells, and HepG2 cells were transfected with a plasmid that expresses luciferase under the control of a TRE promoter. The next day, cells from the four transfected cell cultures were treated with Tet-Express according to the standard protocol and incubated overnight—in parallel with untreated cells from each transfected culture. On the following day, treated and untreated cells were assayed for luciferase activity.

The Lowest Basal Expression of Any TRE-Containing Promoter Available

A pTRE3G vector is included with every Tet-On 3G and Tet-Express system. This vector's inducible promoter (P_{TRE3G}) ensures very low basal expression and high maximal expression after induction (4; see **www.clontech.com/teton3g**). It consists of 7 repeats of a 19 bp *tet* operator sequence located upstream of a minimal CMV promoter, which we refer to as the tetracycline response element (TRE). Although the sequences of the *tet* operator repeats are identical in all Tet system generations, the junction sequences of P_{TRE3G} have been altered to provide an even spacing and the central portions have been randomized. Additionally, elements from the minimal CMV promoter have been mutated to match the consensus sequence (Figure 3).

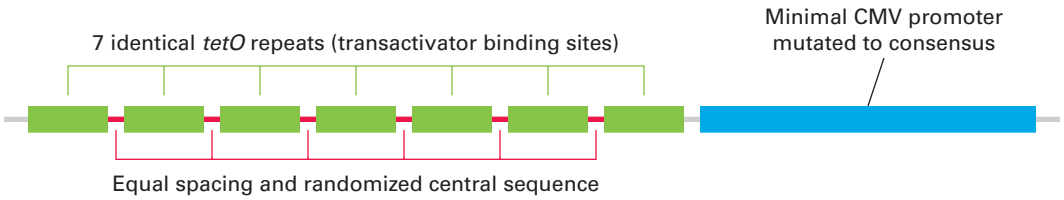

Figure 3. The P_{TRE3G} promoter is designed to ensure the lowest basal expression of any TRE-containing promoter. This promoter consists of 7 identical *tetO* repeats, separated by randomized central sequences of equal length.

Because P_{TRE3G} lacks binding sites for endogenous mammalian transcription factors, and since the minimal CMV promoter is inactive in the absence of a transactivator protein, basal expression from P_{TRE3G} is the lowest of any TRE-containing promoter available so far. Basal expression is undetectable by Western analysis. However, expression can be induced to very high levels in the presence of a transactivator (Figure 4).

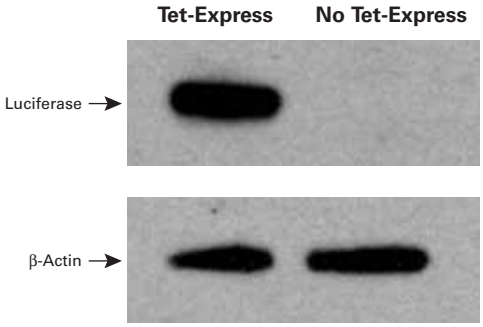

Figure 4. Basal expression from the P_{TRE3G} promoter is undetectable by Western analysis. HeLa cells containing a stably integrated tetracycline-inducible luciferase expression cassette were treated with Tet-Express according to the standard protocol and incubated overnight—in parallel with untreated cells from the same HeLa cell culture. The next day, lysates of treated and untreated cells were prepared and blotted with anti-luciferase and anti-actin antibodies.

Tet-Express...continued

Choice of Four Different Tet-Express System Formats

The Tet-Express Inducible Expression System (which contains the core pTRE3G vector) is also available in three other vector formats. These include the **Tet-Express Inducible Expression System (Bicistronic Version),** the **Tet-Express Inducible Expression System (with mCherry),** and the **Tet-Express Inducible Expression System (with ZsGreen1).** Each is sold as a complete system that provides:

- Tet-Express (25 rxns—includes Tet-Express Transactivator and Intensifier Reagent)
- A vector that expresses your gene of interest under the control of a P_{TRE3G} promoter (the most advanced TRE promoter available)
- A control vector that expresses luciferase under the control of a P_{TRE3G} promoter
- Both hygromycin and puromycin linear selection markers
- Tet Approved FBS
- Xfect™ transfection reagent

System	Vector Map	Applications
Tet-Express Inducible Expression System; Cat. No. 631169	pTRE3G: P_{TRE3G} – MCS – poly A	Get a high level of tightly controlled inducible expression
Tet-Express Inducible Expression System (Bicistronic Version); Cat. No. 631170	pTRE3G-IRES: P_{TRE3G} – MCS – IRES – MCS – poly A	...and inducibly coexpress any two genes of interest
Tet-Express Inducible Expression System (mCherry); Cat. No. 631171	pTRE3G-mCherry: P_{TRE3G} – mCherry – IRES – MCS – poly A	...and monitor inducibility using induced coexpression of the red fluorescent protein mCherry
Tet-Express Inducible Expression System; (ZsGreen1); Cat. No. 631172	pTRE3G-ZsGreen1: P_{TRE3G} – ZsGreen1 – IRES – MCS – poly A	...and monitor inducibility using induced coexpression of the green fluorescent protein ZsGreen1

References

1. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**(12):5547–5551.
2. Gossen, M. *et al.* (1995) *Science* **268**(5218):1766–1769.
3. Urlinger, S. *et al.* (2000) *Proc. Natl. Acad. Sci. USA* **97**(14):7963–7968.
4. Löw, R., Heinz, N., Hampf, M., Bujard, H. & Gossen, M. (2010) *BMC Biotechnology* **10**:81.

Ordering Information

Product	Size	Cat. No.
Tet-Express	25 rxns 100 rxns	631177 631178
Tet-Express Inducible Expression System	each	631169
Tet-Express Inducible Expression System (Bicistronic Version)	each	631170
Tet-Express Inducible Expression System (with mCherry)	each	631171
Tet-Express Inducible Expression System (with ZsGreen1)	each	631172

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