

# pcDNA<sup>™</sup> 5/T0

## USER GUIDE

Hygromycin-resistant expression vector designed for use with  
the T-REx<sup>™</sup> System

**Catalog Number** V103320

**Document Part Number** 25-0407

**Publication Number** MAN0000221

**Revision** A.0



Life Technologies Corporation | 5781 Van Allen Way | Carlsbad, CA 92008

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**Revision history:** Pub. No. MAN0000221

| Revision | Date           | Description               |
|----------|----------------|---------------------------|
| A..0     | 4 January 2019 | Baseline for publication. |

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# Product information

## Introduction

pcDNA<sup>™</sup>5/TO is a 5.7 kb mammalian expression vector that is part of the T-REx<sup>™</sup> Expression System (Cat. Nos. K102001 and K102002). The vector allows tetracycline-regulated expression of the gene of interest in mammalian host cells expressing the Tet repressor (TetR) from the pcDNA<sup>™</sup>6/TR vector (Cat. No. V102520).

The pcDNA<sup>™</sup>5/TO vector contains a hybrid promoter consisting of the human cytomegalovirus immediate-early (CMV) promoter and tetracycline operator 2 (TetO<sub>2</sub>) sites for high-level tetracycline-regulated expression in a wide range of mammalian cells. The vector also contains a hygromycin resistance gene for selection of stable cell lines. A pcDNA<sup>™</sup>5/TO/*lacZ* control plasmid is included as a positive control for transfection and tetracycline-regulated expression in the cell line of choice. For maps and details about these pcDNA<sup>™</sup>5/TO vectors, see page 12.

For more information about pcDNA<sup>™</sup>6/TR and the T-REx<sup>™</sup> Expression System, see the relevant user guides at [thermofisher.com](http://thermofisher.com) (the T-REx<sup>™</sup> Expression System manual is found with any T-REx<sup>™</sup> Complete Kit or T-REx<sup>™</sup> Core Kit).

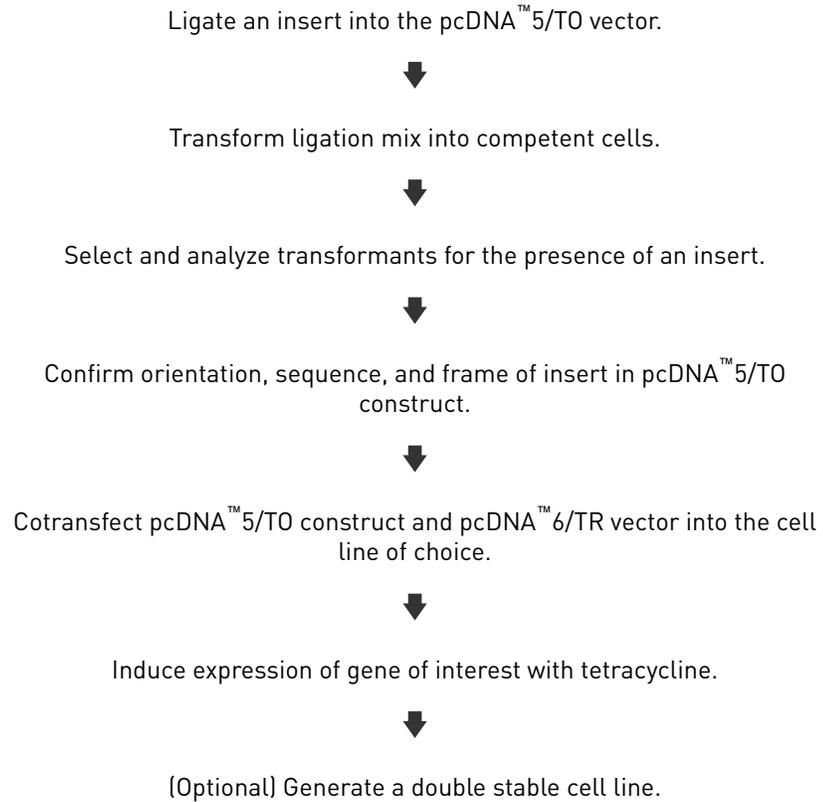
## Contents and storage

The vectors are shipped on wet ice.

| Component                                           | Composition                                                              | Storage |
|-----------------------------------------------------|--------------------------------------------------------------------------|---------|
| pcDNA <sup>™</sup> 5/TO Vector (20 µg)              | 0.5 µg/µL in 40 µL TE buffer, pH 8.0 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) | -20°C   |
| pcDNA <sup>™</sup> 5/TO/ <i>lacZ</i> Vector (20 µg) | 0.5 µg/µL in 40 µL TE buffer, pH 8.0                                     |         |



## Workflow





## Cloning into the pcDNA™ 5/TO vector

Your insert must contain a Kozak consensus sequence with an ATG initiation codon for proper initiation of translation. An example of a Kozak consensus sequence is provided below. Other sequences are possible, but the G or A at position -3 and the G at position +4 (shown in bold) illustrates the most commonly occurring sequence with strong consensus. Replacing one of the two bases at these positions provides moderate consensus, while having neither results in weak consensus. The ATG initiation codon is shown underlined.

(G/A)NNATGG

For a diagram to help you determine how to clone your gene of interest into the pcDNA™ 5/TO vector, see “Multiple cloning site of the pcDNA™ 5/TO vector” on page 8.

### Transformation method

You may use any method of your choice for bacterial transformation. Chemical transformation is the most convenient method for many researchers. Electroporation is the most efficient and the method of choice for large plasmids.

### *E. coli* strain

Many *E. coli* strains are suitable for the propagation and maintenance of this vector including TOP10 and DH5α™-T1<sup>R</sup>. We recommend that you propagate vectors containing inserts in *recA*, *endA* *E. coli* strains.

For your convenience, TOP10 and DH5α™-T1<sup>R</sup> *E. coli* are available as chemically competent or electrocompetent (TOP10 only) cells in a One Shot™ format from Thermo Fisher Scientific.

| Item                                                            | Quantity   | Catalog No. |
|-----------------------------------------------------------------|------------|-------------|
| One Shot™ TOP10 Chemically Competent <i>E. coli</i>             | 21 × 50 µL | C4040-03    |
| One Shot™ TOP10 Electrocomp™ <i>E. coli</i>                     | 21 × 50 µL | C4040-52    |
| One Shot™ MAX Efficiency™ DH5α™-T1 <sup>R</sup> Competent Cells | 21 × 50 µL | 12297-016   |

### Maintenance of the pcDNA™ 5/TO vector

To propagate and maintain the pcDNA™ 5/TO and pcDNA™ 5/TO/*lacZ* vectors, use 10 ng of the vector to transform a *recA*, *endA* *E. coli* strain like TOP10, DH5α™-T1<sup>R</sup>, JM109, or equivalent. Select transformants on LB agar plates containing 50–100 µg/mL ampicillin. Be sure to prepare a glycerol stock of each plasmid for long-term storage (see “Prepare a glycerol stock” on page 8).



## Multiple cloning site of the pcDNA™ 5/TO vector

Below is the multiple cloning site for pcDNA™ 5/TO. Restriction sites are labeled to indicate the cleavage site. Potential stop codons are shown underlined. The multiple cloning site has been confirmed by sequencing and functional testing. To obtain the complete nucleotide sequence for the pcDNA™ 5/TO vector, go to [thermofisher.com](http://thermofisher.com) or contact Technical Support (see page 22). For a map and a description of the features of the pcDNA™ 5/TO vector, see “About the pcDNA™ 5/TO vector” on page 12.

```

721  AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG
                                     CMV Forward priming site
781  GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CCCTATCAGT GATAGAGATC
                                     TATA box
                                     Tetracycline operator (TetO2)
841  TCCCTATCAG TGATAGAGAT CGTCGACGAG CTCGTTTAGT GAACCGTCAG ATCGCCTGGA
                                     Tetracycline operator (TetO2)
901  GACGCCATCC ACGCTGTTTT GACCTCCATA GAAGACACCG GGACCGATCC AGCCTCCGGA
961  CTCTAGCGTT TAAACTTAAAG CTTGGTACCG AGCTCGGATC CACTAGTCCA GTGTGGTGGGA
                                     Pme I*  Afl II  Hind III  Asp718 I  Kpn I          BamH I          BstX I*
1021 ATTCTGCAGA TATCCAGCAC AGTGGCGGCC GCTCGAGTCT AGAGGGCCCG TTTAAACCCG
                                     EcoR V          BstX I*  Not I          Xho I          Xba I          Eco0109 I  Apa I          Pme I*
1081 CTGATCAGCC TCGACTGTGC CTTCTAGTTG CCAGCCATCT
                                     BGH Reverse priming site

```

\*Please note that there are two *Pme* I sites and two *BstX* I sites in the polylinker.

## *E. coli* transformation

Transform your ligation mixtures into a competent *recA*, *endA* *E. coli* strain (e.g. TOP10, DH5α™-T1<sup>R</sup>) and select on LB agar plates containing 50–100 µg/mL ampicillin. Select 10–20 clones and analyze for the presence and orientation of your insert.

**Note:** We recommend that you sequence your construct to confirm that your gene is in the correct orientation for expression and contains an initiation ATG and a stop codon. See “Multiple cloning site of the pcDNA™ 5/TO vector” on page 8 for the sequence and location of recommended primer binding sites.

A custom primer synthesis service is available from Thermo Fisher Scientific for your convenience. For more information, go to [thermofisher.com](http://thermofisher.com) or contact Technical Support (see page 22).

## Prepare a glycerol stock

Once you have identified the correct clone, purify the colony and make a glycerol stock for long-term storage. You should keep a DNA stock of your plasmid at –20°C.

- Streak the original colony out on an LB plate containing 50 µg/mL ampicillin. Incubate the plate at 37°C overnight.
- Isolate a single colony and inoculate into 1–2 mL of LB containing 50 µg/mL ampicillin.
- Grow the culture to mid-log phase (OD<sub>600</sub> = 0.5–0.7).
- Mix 0.85 mL of culture with 0.15 mL of sterile glycerol and transfer to a cryovial.
- Store at –80°C.



## Transfection

Once you have cloned your gene of interest into pcDNA<sup>™</sup> 5/TO and have prepared clean plasmid preparations of your pcDNA<sup>™</sup> 5/TO construct and pcDNA<sup>™</sup> 6/TR, you are ready to cotransfect the plasmids into the mammalian cell line of choice. We recommend that you include the positive control vector and a mock transfection (negative control) to evaluate your results. Refer to the T-REx<sup>™</sup> Expression System manual for information on pcDNA<sup>™</sup> 6/TR, transfection, and induction of expression using tetracycline.

### Plasmid preparation

Plasmid DNA for transfection into eukaryotic cells must be very clean and free from phenol and sodium chloride. Contaminants will kill the cells, and salt will interfere with lipid complexing, decreasing transfection efficiency. We recommend isolating plasmid DNA using the S.N.A.P.<sup>™</sup> Miniprep Kit (10–15 µg DNA, Catalog No. K1900-01), the S.N.A.P.<sup>™</sup> MidiPrep Kit (10–200 µg DNA, Catalog No. K1910-01), or CsCl gradient centrifugation.

### Positive controls

pcDNA<sup>™</sup> 5/TO/*lacZ* is provided as a positive control vector for mammalian cell transfection and expression (see page 14) and may be used to optimize transfection conditions for your cell line. Cotransfection of the positive control vector and pcDNA<sup>™</sup> 6/TR results in the induction of β-galactosidase expression upon addition of tetracycline. A successful cotransfection will result in β-galactosidase expression that can be easily assayed by staining with X-gal (see “Assay for β-galactosidase activity” on page 16).

### Cotransfection and induction with tetracycline

The following general guidelines are provided to cotransfect your pcDNA<sup>™</sup> 5/TO construct (or the control plasmid) and pcDNA<sup>™</sup> 6/TR into your cell line of interest, and to induce expression of your protein of interest with tetracycline.

- Use cells that are approximately 60% confluent for transfection.
- Cotransfect your pcDNA<sup>™</sup> 5/TO construct and pcDNA<sup>™</sup> 6/TR at a ratio of 6:1 (w:w) into the cell line of choice using your preferred method. Absolute<sup>™</sup> amounts of plasmid used for transfection will vary depending on the method of transfection and the cell line used.
- After transfection, add fresh medium and allow the cells to recover for 24 hours before induction.
- Remove medium and add fresh medium containing the appropriate concentration of tetracycline to the cells. We recommend that you add tetracycline to a final concentration of 1µg/mL to the cells and incubate the cells for 24 hours at 37°C.
- Harvest the cells and assay for expression of your gene of interest.



## Stable cell line generation

Once it is established that your construct can be inducibly expressed, you can create a stable cell line that inducibly expresses your gene of interest. The pcDNA™ 5/TO vector contains the hygromycin resistance gene for selection of stable transfectants with the antibiotic, hygromycin B. When added to cultured mammalian cells, hygromycin B acts as an aminocyclitol to inhibit protein synthesis to select against cells that do not carry your construct.

When generating a stable cell line expressing the Tet repressor (from pcDNA™ 6/TR), **always select for clones that express the highest levels of Tet repressor to use as hosts for your pcDNA™ 5/TO expression plasmid.** Clones which express the highest levels of Tet repressor should also exhibit the most complete repression of basal transcription of your gene of interest.

**Note:** Your gene of interest is constitutively expressed when the pcDNA™ 5/TO construct is transfected into mammalian host cells prior to transfecting the pcDNA™ 6/TR plasmid. For more information on selection of stable cell lines using pcDNA™ 6/TR and blasticidin, refer to the T-REx™ Expression System manual.

### Determine antibiotic sensitivity

To generate a stable cell line expressing your protein of interest, determine the minimum concentration of hygromycin required to kill your untransfected host cell line. Typically, concentrations between 10 and 400 µg/mL hygromycin are sufficient to kill the untransfected host cell line. Test a range of concentrations (see below) to ensure that you determine the minimum concentration necessary for your cell line.

**Note:** Before transfecting your host cell line with pcDNA™ 6/TR, you will need to perform a similar experiment to determine the minimum concentration of blasticidin required to kill the untransfected cell line. Refer to the T-REx™ System manual for information about blasticidin.

- Plate or split a confluent plate so the cells will be approximately 25% confluent. Prepare a set of 7 plates.
- The next day, substitute culture medium with medium containing varying concentrations of hygromycin (e.g., 0, 10, 25, 50, 100, 200, and 400 µg/mL).
- Replenish the selective medium every 3–4 days, and observe the percentage of surviving cells.
- Count the number of viable cells at regular intervals to determine the appropriate concentration of hygromycin that prevents growth within 1–2 weeks after addition of hygromycin.



## Possible sites for linearization

To obtain stable transfectants, you have the option to linearize your pcDNA™ 5/TO construct before transfection. While linearizing your vector may not improve the efficiency of transfection, it increases the chances that the vector does not integrate in a way that disrupts either the gene of interest or other elements important for mammalian expression. The table below lists unique sites that may be used to linearize your construct prior to transfection. Other restriction sites are possible. **Be sure that your insert does not contain the restriction enzyme site you want to use to linearize your vector.**

| Enzyme            | Restriction Site (bp) | Location                 | Supplier                                |
|-------------------|-----------------------|--------------------------|-----------------------------------------|
| <i>Mun</i> I      | 162                   | Upstream of CMV promoter | ER0752                                  |
| <i>Nru</i> I      | 209                   | Upstream of CMV promoter | ER0111                                  |
| <i>Sap</i> I      | 3736                  | Backbone                 | ER1932                                  |
| <i>Eam</i> 1105 I | 4745                  | Ampicillin gene          | AGS <sup>[1]</sup> , Fermentas, Takara™ |
| <i>Fsp</i> I      | 4967                  | Ampicillin gene          | ER1221                                  |
| <i>Ssp</i> I      | 5549                  | <i>bla</i> promoter      | ER0772                                  |

[1] Angewandte Gentechnologie Systeme

## Select stable cell lines

Once you have determined the appropriate hygromycin concentration to use for selection, you can generate a stable cell line expressing pcDNA™ 6/TR and your pcDNA™ 5/TO construct.

1. Cotransfect your pcDNA™ 5/TO construct and pcDNA™ 6/TR into the cell line of choice using your preferred method. Include a sample of untransfected cells as a negative control.
2. 24 hours after transfection, wash the cells and add fresh medium to the cells.
3. 48 hours after transfection, split the cells into fresh medium containing hygromycin and blasticidin at the appropriate concentration for your cell line. Split the cells such that they are no more than 25% confluent. If the cells are too dense, the antibiotic will not kill the untransfected cells.
4. Replenish selective medium every 3–4 days until hygromycin-resistant and blasticidin-resistant colonies are detected.
5. Pick at least 40 foci and expand them to test for tetracycline-inducible gene expression.

**Note:** You may also create stable cell lines by first stably transfecting the pcDNA™ 6/TR plasmid into your cell line of choice, and then using this cell line as the host for your pcDNA™ 5/TO construct. If you are using one of the T-REx™ cell lines, simply transfect your pcDNA™ 5/TO construct into the cells and select for hygromycin-resistant and blasticidin-resistant clones.



# Vector information

## About the pcDNA<sup>™</sup> 5/TO vector

The pcDNA<sup>™</sup> 5/TO vector contains two tetracycline operator 2 (TetO<sub>2</sub>) sites within the human cytomegalovirus immediate-early (CMV) promoter for tetracycline-regulated expression of your gene of interest. The TetO<sub>2</sub> sequences serve as binding sites for 4 Tet repressor molecules (two Tet repressor homodimers) and confer tetracycline-responsiveness to your gene of interest. The Tet repressor is expressed from the pcDNA<sup>™</sup> 6/TR plasmid. For more information about the TetO<sub>2</sub> sequences, see the “About Tet operator sequences” on page 12. For more information about the pcDNA<sup>™</sup> 6/TR plasmid and the Tet repressor, refer to the T-REx<sup>™</sup> Expression System manual.

In the absence of tetracycline, expression of your gene of interest is repressed by the binding of Tet repressor homodimers to the TetO<sub>2</sub> sequences. Addition of tetracycline to the cells derepresses the hybrid CMV/TetO<sub>2</sub> promoter in pcDNA<sup>™</sup> 5/TO and allows expression of your gene of interest.

### About Tet operator sequences

The promoters of bacterial *tet* genes contain two types of operator sequences, O<sub>1</sub> and O<sub>2</sub>, that serve as high affinity binding sites for the Tet repressor. Each O<sub>1</sub> and O<sub>2</sub> site binds to one Tet repressor homodimer. While Tet repressor homodimers bind to both *tet* operators with high affinity, studies have shown that the affinity of the Tet repressor homodimer for O<sub>2</sub> is three- to five-fold higher than it is for O<sub>1</sub>.

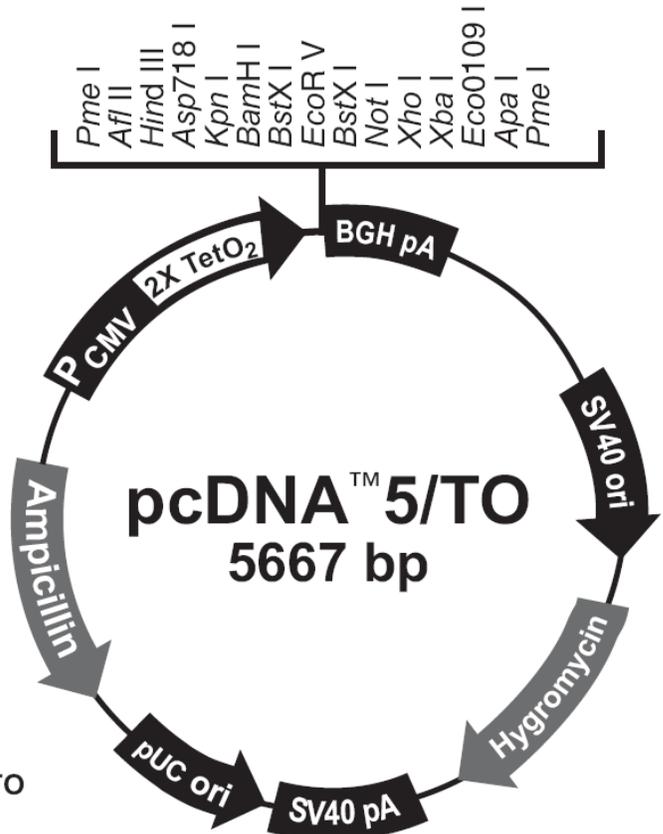
*Tet* operators have been incorporated into heterologous eukaryotic promoters to allow tetracycline-regulated gene expression in mammalian cells. In the T-REx<sup>™</sup> Expression System, two copies of the O<sub>2</sub> operator sequence (TetO<sub>2</sub>) are inserted into the strong CMV promoter of pcDNA<sup>™</sup> 5/TO to allow regulated expression of your gene of interest by tetracycline. We use the TetO<sub>2</sub> operator sequence in pcDNA<sup>™</sup> 5/TO to maximize repression of basal gene expression.

The location of *tet* operator sequences in relation to the TATA box of a heterologous promoter is critical to the function of the *tet* operator. Regulation by tetracycline is only conferred upon a heterologous promoter by proper spacing of the TetO<sub>2</sub> sequences from the TATA box. For this reason, the first nucleotide of the TetO<sub>2</sub> operator sequence has been placed 10 nucleotides after the last nucleotide of the TATA element in the CMV promoter in pcDNA<sup>™</sup> 5/TO. Refer to the “Multiple cloning site of the pcDNA<sup>™</sup> 5/TO vector” on page 8 for the sequence and placement of the TetO<sub>2</sub> sequences in relation to the TATA box.

In other tetracycline-regulated systems, the TetO<sub>2</sub> sequences are located upstream of the TATA element in the promoter of the inducible expression vector. These systems differ substantially from the T-REx<sup>™</sup> Expression System in that they use regulatory molecules composed of the Tet repressor fused to a viral transactivation domain. The presence of viral transactivation domains appears to overcome the requirement for specific positioning of the TetO<sub>2</sub> sequences in relation to the TATA box of the heterologous promoter. However, the presence of viral transactivation domains has been found to have deleterious effects in some mammalian cell lines.

## Map of the pcDNA™ 5/TO vector

The following map summarizes the features of the pcDNA™ 5/TO vector. To obtain the complete nucleotide sequence for the pcDNA™ 5/TO vector, go to [thermofisher.com](http://thermofisher.com) or contact Technical Support (see page 22).



### Comments for pcDNA™ 5/TO 5667 nucleotides

CMV promoter: bases 232-958

TATA box: bases 804-810

Tetracycline operator (2X TetO<sub>2</sub>) sequences: bases 820-859

CMV forward priming site: bases 769-789

Multiple cloning site: bases 968-1077

BGH reverse priming site: bases 1089-1106

BGH polyadenylation sequence: bases 1095-1319

SV40 promoter and origin: bases 1820-2128

Hygromycin resistance gene: bases 2185-3210

SV40 early polyadenylation sequence: bases 3340-3470

pUC origin: bases 3853-4526 (complementary strand)

*bla* promoter: bases 5532-5630 (complementary strand)

Ampicillin (*bla*) resistance gene: bases 4671-5531 (complementary strand)

## Features of the pcDNA™ 5/TO vector

pcDNA™ 5/TO is a 5667 bp vector that expresses your gene of interest under the control of a hybrid CMV/TetO<sub>2</sub> promoter. The table below describes the relevant features of pcDNA™ 5/TO. All features have been functionally tested.

| Feature                                                             | Benefit                                                                                                                                                        |
|---------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Human cytomegalovirus (CMV) immediate early promoter                | Allows high-level expression of your gene of interest (Andersson <i>et al.</i> , 1989; Boshart <i>et al.</i> , 1985; Nelson <i>et al.</i> , 1987)              |
| CMV Forward priming site                                            | Allows sequencing in the sense orientation                                                                                                                     |
| Tetracycline operator (O <sub>2</sub> ) sequences                   | Two tandem 19 nucleotide repeats which serve as binding sites for Tet repressor homodimers (Hillen and Berens, 1994)                                           |
| Multiple cloning site                                               | Allows insertion of your gene of interest                                                                                                                      |
| BGH Reverse priming site                                            | Allows sequencing of the non-coding strand                                                                                                                     |
| Bovine growth hormone (BGH) polyadenylation signal                  | Allows efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)                                                             |
| SV40 early promoter and origin                                      | Allows efficient, high-level expression of the hygromycin resistance gene in mammalian cells and episomal replication in cells expressing SV40 large T antigen |
| Hygromycin resistance gene (expressed from the SV40 early promoter) | Allows selection of stable transfectants in mammalian cells (Gritz and Davies, 1983)                                                                           |
| SV40 early polyadenylation signal                                   | Allows efficient transcription termination and polyadenylation of mRNA                                                                                         |
| pUC origin                                                          | Allows high-copy number replication and growth in <i>E. coli</i>                                                                                               |
| <i>bla</i> promoter                                                 | Allows expression of the ampicillin ( <i>bla</i> ) resistance gene                                                                                             |
| Ampicillin ( <i>bla</i> ) resistance gene (β-lactamase)             | Allows selection of transformants in <i>E. coli</i>                                                                                                            |

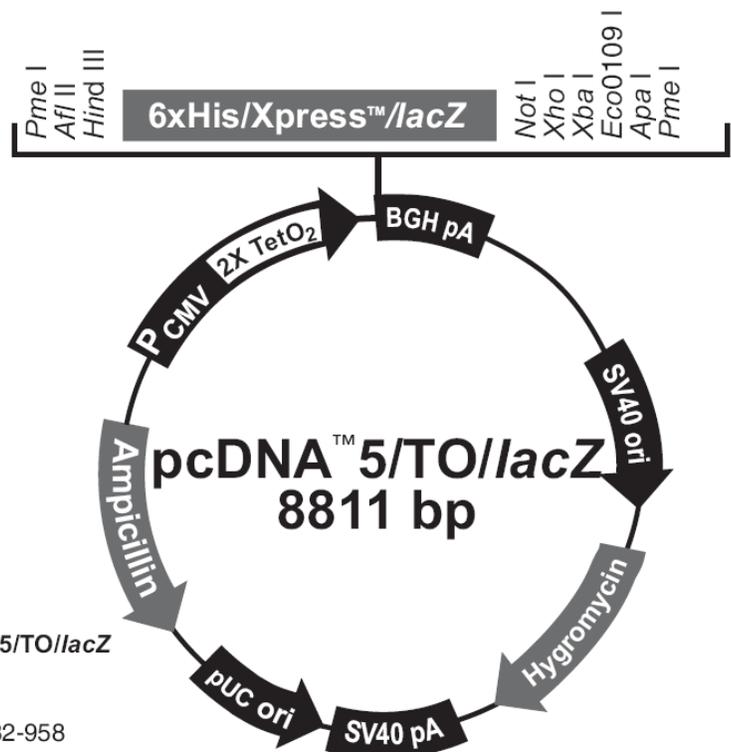
## About the pcDNA™ 5/TO/lacZ vector

pcDNA™ 5/TO/lacZ is a 8811 bp control vector containing the gene for β-galactosidase. This vector was constructed by ligating a 3.1 kb *Hind* III-*Pst* I fragment containing the *lacZ* gene from pcDNA™ 3/His/lacZ into the *Hind* III-*Pst* I site of pcDNA™ 5/TO.

**Note:** The *lacZ* gene is fused to an N-terminal peptide containing an ATG initiation codon, a polyhistidine (6xHis) tag, and the Xpress™ epitope. The size of the β-galactosidase fusion protein is approximately 120 kDa in size.

### Map of the pcDNA™ 5/TO/lacZ vector

The following map summarizes the features of the pcDNA™ 5/TO/lacZ vector. To obtain the complete nucleotide sequence for the pcDNA™ 5/TO/lacZ vector, go to [thermofisher.com](http://thermofisher.com) or contact Technical Support (see page 22).



#### Comments for pcDNA™ 5/TO/lacZ 8811 nucleotides

CMV promoter: bases 232-958

TATA box: bases 804-810

Tetracycline operator (2X TetO<sub>2</sub>) sequences: bases 820-859

CMV forward priming site: bases 769-789

Polyhistidine (6xHis) tag: bases 999-1016

Xpress™ epitope: bases 1056-1079

*LacZ* ORF: bases 1104-4154

BGH reverse priming site: bases 4235-4252

BGH polyadenylation sequence: bases 4241-4465

SV40 promoter and origin: bases 4966-5275

Hygromycin resistance gene: bases 5332-6357

SV40 early polyadenylation sequence: bases 6487-6617

pUC origin: bases 7000-7673 (complementary strand)

*bla* promoter: bases 8676-8774 (complementary strand)

Ampicillin (*bla*) resistance gene: bases 7815-8675 (complementary strand)



# Detection of $\beta$ -galactosidase expression

## Assay for $\beta$ -galactosidase activity

You may assay for  $\beta$ -galactosidase expression by activity assay using cell-free lysates or by staining the cells for activity. Reagents for fast and easy detection of  $\beta$ -galactosidase expression are available from Thermo Fisher Scientific (see page 21).

**Note:** The *lacZ* gene in pcDNA<sup>™</sup>5/TO/*lacZ* is fused to an N-terminal peptide containing an ATG initiation codon, a 6xHis tag and the Xpress<sup>™</sup> epitope. The Xpress<sup>™</sup> epitope allows detection of the  $\beta$ -galactosidase fusion protein on a western blot using the Xpress<sup>™</sup> Antibody. The N-terminal peptide adds approximately 4.3 kDa to the size of the  $\beta$ -galactosidase fusion protein (total size of the fusion protein is approximately 120 kDa).

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**IMPORTANT!** Because tetracycline-regulated expression in the T-REx<sup>™</sup> System is based on a repression/derepression mechanism, the amount of Tet repressor that is expressed in the host cell line from pcDNA<sup>™</sup>6/TR will determine the level of transcriptional repression of the Tet operator sequences in your pcDNA<sup>™</sup>5/TO construct. Tet repressor levels should be sufficiently high to suitably repress basal level transcription. We have varied the ratio of pcDNA<sup>™</sup>6/TR and pcDNA<sup>™</sup>5/TO plasmid that we transiently cotransfect into mammalian cells to optimize repression and inducibility of the hybrid CMV/TetO<sub>2</sub> promoter in pcDNA<sup>™</sup>5/TO. We recommend that you cotransfect your mammalian host cell line with a ratio of **at least 6:1** (w/w) pcDNA<sup>™</sup>6/TR:pcDNA<sup>™</sup>5/TO plasmid DNA, but you may want to try varying ratios of pcDNA<sup>™</sup>6/TR:pcDNA<sup>™</sup>5/TO plasmid to optimize repression and expression for your particular cell line and your gene of interest.

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## Detection of $\beta$ -Galactosidase fusion proteins

To detect expression of the  $\beta$ -galactosidase fusion protein from pcDNA<sup>™</sup>5/TO/*lacZ* by western blot, use the Xpress<sup>™</sup> Antibody available from Thermo Fisher Scientific (see page 22 for ordering information). The antibody should detect a  $\beta$ -galactosidase fusion protein of approximately 120 kDa in size. To perform a western blot, you will need to prepare a cell lysate from transfected cells. A sample protocol is provided below. Other protocols are suitable.

## Prepare cell lysis buffer

1. Prepare cell lysis buffer from the following common stock solutions. For 100 mL, combine:

| Reagent       | Volume | Final concentration |
|---------------|--------|---------------------|
| 1 M Tris-HCl  | 5 mL   | 50 mM Tris-HCl      |
| 5 M NaCl      | 3 mL   | 150 mM NaCl         |
| Nonidet™ P-40 | 1 mL   | 1% Nonidet™ P-40    |

2. Bring the volume up to 90 mL with deionized water and adjust the pH to 7.8 with HCl.
3. Bring the volume up to 100 mL. Store at room temperature.

**Note:** Protease inhibitors may be added at the following concentrations: 1 mM PMSF, 1  $\mu$ g/mL pepstatin, and 1  $\mu$ g/mL leupeptin

## Prepare cell lysates

Use the following protocol to prepare cell lysates.

1. Wash cells ( $\sim 10^6$  cells) once with phosphate-buffered saline (PBS).
2. Scrape cells into 1 mL PBS and centrifuge the cells at  $1500 \times g$  for 5 minutes.
3. Resuspend the cell pellet in 50  $\mu$ L Cell Lysis Buffer. Other cell lysis buffers are suitable.
4. Incubate cell suspension at 37°C for 10 minutes to lyse the cells.

**Note:** Cell lysis can also be performed on ice or at room temperature.

5. Centrifuge the cell lysate at  $10,000 \times g$  for 10 minutes to pellet nuclei, then transfer the supernatant to a fresh tube. Assay the lysate for protein concentration.

**Note:** Do not use protein assays utilizing Coomassie™ Blue or other dyes. NP-40 interferes with the binding of the dye with the protein.

6. Add SDS-PAGE sample buffer to a final concentration of 1X and boil the sample for 5 minutes.
7. Load 20  $\mu$ g of lysate onto an SDS-PAGE gel with the appropriate percentage of acrylamide to resolve your fusion protein.



# Safety



**WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
  - Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.
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## Chemical safety



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**WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
  - Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
  - Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
  - Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
  - Handle chemical wastes in a fume hood.
  - Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
  - After emptying a waste container, seal it with the cap provided.
  - Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
  - Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
  - **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.
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## Biological hazard safety



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**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:  
[www.cdc.gov/biosafety/publications/bmb15/BMBL.pdf](http://www.cdc.gov/biosafety/publications/bmb15/BMBL.pdf)
  - World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:  
[www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf](http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf)
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# Accessory products

## Accessory products

The products listed below are designed for use with the T-REx™ Expression System. For detailed information, visit our website at [thermofisher.com](http://thermofisher.com) or call Technical Support.

### T-REx™ cell lines

Several mammalian cell lines that stably express the Tet repressor are available for your convenience. T-REx™-293 cells, T-REx™-HeLa cells, T-REx™-CHO cells, and T-REx™-Jurkat cells express the Tet repressor from pcDNA™6/TR and should be maintained in medium containing blasticidin. Expression of your gene of interest from pcDNA™5/TO may be assayed by transfection of your pcDNA™5/TO construct into any of the T-REx™ cell lines and induction with tetracycline. Ordering information is provided below.

| Cell Line     | Source                        | Catalog No. |
|---------------|-------------------------------|-------------|
| T-REx™-293    | Human embryonic kidney        | R710-07     |
| T-REx™-HeLa   | Human cervical adenocarcinoma | R714-07     |
| T-REx™-CHO    | Chinese hamster ovary         | R718-07     |
| T-REx™-Jurkat | Human lymphocyte              | R722-07     |

### Additional reagents

Additional reagents included in, or used in conjunction with the T-REx™ Expression System are available separately from Thermo Fisher Scientific.

| Item                        | Amount        | Catalog No. |
|-----------------------------|---------------|-------------|
| pcDNA™6/TR                  | 20 µg         | V1025-20    |
| pcDNA™4/TO                  | 20 µg         | V1020-20    |
| pcDNA™4/myc-His A, B, and C | 20 µg each    | V1030-20    |
| Blasticidin                 | 50 mg, powder | R210-01     |
| β-Gal Assay Kit             | 80 mL         | K1455-01    |
| β-Gal Staining Kit          | 1 kit         | K1465-01    |
| Xpress™ Antibody [1]        | 50 µL         | R910-25     |

[1] The amount of antibody supplied is sufficient to detect 25 western blots in a 10 mL working volume.



# Documentation and support

## Customer and technical support

Visit [thermofisher.com/support](http://www.thermofisher.com/support) for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

**Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

## Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at [www.thermofisher.com/us/en/home/global/terms-and-conditions.html](http://www.thermofisher.com/us/en/home/global/terms-and-conditions.html). If you have any questions, please contact Life Technologies at [www.thermofisher.com/support](http://www.thermofisher.com/support).



