invitrogen™

pcDNA™ 5/FRT/TO

Inducible expression vector designed for use with the Flp-In™ T-REx™ System

Cat. no. V6520-20

Version G
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25-0368

User Manual

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Kit Contents and Storage

Contents

20 µg of pcDNA™5/FRT/TO in TE buffer, pH 8.0 (40 µl at 0.5 µg/µl)

20 µg of pcDNA™5/FRT/TO/CAT in TE buffer, pH 8.0 (40 µl at 0.5 µg/µl)

TE Buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0

Shipping/Storage

Plasmids are supplied in TE buffer and shipped on wet ice. They should be stored at –20°C upon arrival.

Accessory Products

Many of the reagents used in the Flp-In™ T-REx™ System are available separately from Invitrogen. See the table below for ordering information.

Item	Amount	Cat. no.
Zeocin™	1 g	R250-01
	5 g	R250-05
pFRT/*lac*Zeo	20 µg in TE, pH 8.0 (40 µl at 0.5 µg/µl)	V6015-20
pFRT/*lac*Zeo2	20 µg in TE, pH 8.0 (40 µl at 0.5 µg/µl)	V6022-20
pcDNA™6/TR	20 µg in TE, pH 8.0 (40 µl at 0.5 µg/µl)	V1025-20
pOG44	20 µg in TE, pH 8.0 (40 µl at 0.5 µg/µl)	V6005-20
PureLink™ HQ Plasmid Miniprep Kit	100 reactions	K2100-01

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Kit Contents and Storage, continued

Other Flp-In™ T-REX™ Products

A number of other Flp-In™ T-REx™ products are available from Invitrogen to facilitate expression of your gene of interest in the Flp-In™ T-REx™ System. The Flp-In™ T-REx™ Core Kit contains vectors (pFRT/*lac*Zeo, pcDNA™6/TR, pcDNA™5/FRT/TO, and pOG44), primers, and tetracycline.
The pcDNA™5/FRT/TO TOPO® TA Expression Kit allows rapid and efficient TOPO® Cloning of *Taq*-amplified PCR products into the pcDNA™5/FRT/TO-TOPO© vector. The Flp-In™ T-REx™-293 Cell Line contains a single integrated FRT site and stably expresses the Tet repressor, and allows the user to proceed directly to generation of the Flp-In™ T-REx™ expression cell line.
For more information about these products go to www.invitrogen.com or contact Technical Support (see page 12).

Cell Line	Amount	Cat. no.
Flp-In™ T-REx™ Core Kit	1 kit	K6500-01
pcDNA™5/FRT/TO TOPO® TA Expression Kit	20 reactions	K6510-20
Flp-In™ T-REx™-293	3×10^6 cells, frozen	R780-07

Flp-In™ Host Cell Lines

For your convenience, Invitrogen has available several mammalian Flp-In™ host cell lines that stably express the *lacZ-Zeocin*™ fusion gene from pFRT/*lac*Zeo or pFRT/*lac*Zeo2 (Flp-In™-CHO). Each cell line contains a single integrated FRT site as confirmed by Southern blot analysis. By transfecting the pcDNA™6/TR plasmid into these cell lines, you can easily generate Flp-In™ T-REx™ host cell lines.
For more information go to www.invitrogen.com or contact Technical Support (see page 12).

Cell Line	Amount	Cat. no.
Flp-In™-293	3×10^6 cells, frozen	R750-07
Flp-In™-CV-1	3×10^6 cells, frozen	R752-07
Flp-In™-CHO	3×10^6 cells, frozen	R758-07

Methods

Overview

Introduction

pcDNA™5/FRT/TO is a 5.1 kb inducible expression vector designed for use with the Flp-In™ T-REx™ System (Cat. no. K6500-01) available from Invitrogen. When cotransfected with the pOG44 Flp recombinase expression plasmid into a Flp-In™ T-REx™ mammalian host cell line, the pcDNA™5/FRT/TO vector containing the gene of interest is integrated in a Flp recombinase-dependent manner into the genome. Expression of the gene of interest may be induced by the addition of tetracycline to the culture medium. The vector contains the following elements:

- A hybrid human cytomegalovirus (CMV)/$TetO_2$ promoter for high-level, tetracycline-regulated expression of the gene of interest in a wide range of mammalian cells (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Hillen and Berens, 1994; Hillen *et al.*, 1983; Nelson *et al.*, 1987)
- Multiple cloning site with 10 unique restriction sites to facilitate cloning the gene of interest
- FLP Recombination Target (FRT) site for Flp recombinase-mediated integration of the vector into the Flp-In™ T-REx™ host cell line (see page 2 for more information)
- Hygromycin resistance gene for selection of stable cell lines (Gritz and Davies, 1983)

The control plasmid, pcDNA™5/FRT/TO/CAT, is included for use as a positive control for transfection and expression in the Flp-In™ T-REx™ host cell line of choice.

For more information about the Flp-In™ T-REx™ System, the pOG44 plasmid, and generation of the Flp-In™ T-REx™ host cell line, refer to the Flp-In™ T-REx™ Core Kit manual. The Flp-In™ T-REx™ Core Kit manual is supplied with the Flp-In™ T-REx™ Core Kit, but is also available from www.invitrogen.com or by contacting Technical Support (see page 12).

Hybrid CMV/$TetO_2$ Promoter

Expression of your gene of interest from pcDNA™5/FRT/TO is controlled by the strong CMV immediate early enhancer/promoter (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987) into which 2 copies of the *tet* operator 2 ($TetO_2$) sequence have been inserted in tandem. Insertion of these $TetO_2$ sequences into the CMV promoter confers regulation by tetracycline to the promoter.

The $TetO_2$ sequences consist of 2 copies of the 19 nucleotide sequence, 5´-TCCCTATCAGTGATAGAGA-3´ separated by a 2 base pair spacer (Hillen and Berens, 1994; Hillen *et al.*, 1983). Each 19 nucleotide $TetO_2$ sequence serves as the binding site for 2 molecules of the Tet repressor. For more information about the mechanism of tetracycline regulation in the Flp-In™ T-REx™ System, refer to the Flp-In™ T-REx™ Core Kit manual.

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Overview, continued

A Note About pcDNA™5/FRT/TO

The pcDNA™5/FRT/TO vector contains a single FRT site immediately upstream of the hygromycin resistance gene for Flp recombinase-mediated integration and selection of the pcDNA™5/FRT/TO plasmid following cotransfection of the vector (with pOG44) into Flp-In™ T-REx™ mammalian host cells. The FRT site serves as both the recognition and cleavage site for the Flp recombinase and allows recombination to occur immediately adjacent to the hygromycin resistance gene. The Flp recombinase is expressed from the pOG44 plasmid. For more information about the FRT site and recombination, see the next page. For more information about pOG44, refer to the Flp-In™ T-REx™ Core Kit manual.

Important

The hygromycin resistance gene in pcDNA™5/FRT/TO lacks a promoter and an ATG initiation codon; therefore, transfection of the pcDNA™5/FRT/TO plasmid alone into mammalian host cells will **not** confer hygromycin resistance to the cells. The SV40 promoter and ATG initiation codon required for expression of the hygromycin resistance gene are integrated into the genome (in the Flp-In™ T-REx™ host cell line) and are only brought into the correct proximity and frame with the hygromycin resistance gene through Flp recombinase-mediated integration of pcDNA™5/FRT/TO at the FRT site. For more information about the generation of the Flp-In™ T-REx™ host cell line and details of the Flp-In™ T-REx™ System, refer to the Flp-In™ T-REx™ Core Kit manual.

Flp Recombinase-Mediated DNA Recombination

In the Flp-In™ T-REx™ System, integration of your pcDNA™5/FRT/TO inducible expression construct into the genome occurs via Flp recombinase-mediated intermolecular DNA recombination. The hallmarks of Flp-mediated recombination are listed below.

- Recombination occurs between specific FRT sites (see below) on the interacting DNA molecules
- Recombination is conservative and requires no DNA synthesis; the FRT sites are preserved following recombination and there is minimal opportunity for introduction of mutations at the recombination site
- Strand exchange requires only the small 34 bp minimal FRT site (see next page)

For more information about the Flp recombinase and conservative site-specific recombination, refer to published reviews (Craig, 1988; Sauer, 1994).

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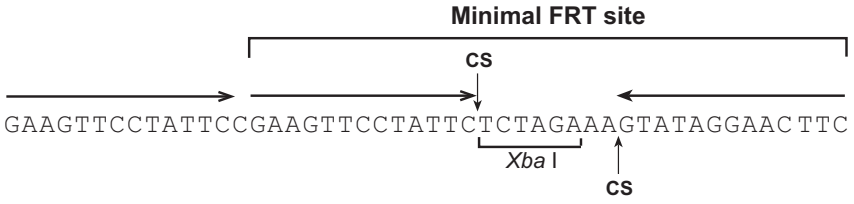
Overview, continued

FRT Site

The FRT site, originally isolated from *Saccharomyces cerevisiae*, serves as a binding site for Flp recombinase and has been well-characterized (Gronostajski and Sadowski, 1985; Jayaram, 1985; Sauer, 1994; Senecoff *et al.*, 1985). The minimal FRT site consists of a 34 bp sequence containing two 13 bp imperfect inverted repeats separated by an 8 bp spacer that includes an *Xba* I restriction site (see figure below). An additional 13 bp repeat is found in most FRT sites, but is not required for cleavage (Andrews *et al.*, 1985). While Flp recombinase binds to all three of the 13 bp repeats, strand cleavage actually occurs at the boundaries of the 8 bp spacer region (see figure below) (Andrews *et al.*, 1985; Senecoff *et al.*, 1985).



CS = cleavage site

Experimental Outline

The following table outlines the steps required to clone and inducibly express your gene of interest in pcDNA™5/FRT/TO.

Step	Action
1	Consult the multiple cloning site diagrammed on page 5 to design your cloning strategy.
2	Ligate your insert into pcDNA™5/FRT/TO and transform into *E. coli*. Select transformants on 50 to 100 µg/ml ampicillin.
3	Analyze your transformants for the presence of insert by restriction digestion.
4	Select a transformant with the correct restriction pattern and sequence to confirm that your gene is cloned in the correct orientation.
5	Cotransfect your pcDNA™5/FRT/TO construct and pOG44 into the Flp-In™ T-REx™ host cell line using your own method of choice and select for hygromycin resistant clones (see the Flp-In™ T-REx™ Core Kit manual for more information).
6	Add tetracycline to induce expression of the gene of interest (see the Flp-In™ T-REx™ Core Kit manual for more information).
7	Assay for expression of the gene of interest.

Cloning into pcDNA™5/FRT/TO

Introduction

A diagram is provided on the next page to help you clone your gene of interest into pcDNA™5/FRT/TO. General considerations for cloning and transformation are listed below.

General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, DNA sequencing, and DNA biochemistry, refer to *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

E. coli Strain

Many *E. coli* strains are suitable for the propagation and maintenance of this vector. We recommend that you propagate vectors containing inserts in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease A deficient (*end*A).

For your convenience, TOP10 and DH5α™-T1^R cells are available as chemically competent or electrocompetent (TOP10 only) cells from Invitrogen.

Item	Quantity	Cat. no.
One Shot® TOP10 Chemically Competent Cells	20 reactions	C4040-03
One Shot® TOP10 Electrocomp™ Cells	20 reactions	C4040-52
One Shot® DH5α™-T1^R Max Efficiency® Chemically Competent Cells	20 reactions	12297-016

Transformation Method

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

Maintenance of Plasmids

To propagate and maintain the pcDNA™5/FRT/TO and pcDNA™5/FRT/TO/CAT vectors, use 10 ng of each vector to transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α™-T1^R, JM109, or equivalent. Select transformants on LB agar plates containing 50 to 100 µg/ml ampicillin. Be sure to prepare a glycerol stock of each plasmid for long-term storage (see page 6).

continued on next page

Cloning into pcDNA™5/FRT/TO, continued

Cloning Considerations

Your insert should contain a Kozak consensus sequence with an ATG initiation codon for proper initiation of translation (Kozak, 1987; Kozak, 1991; Kozak, 1990). 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) illustrate 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)NNATG**G**

Your insert must also contain a stop codon for proper termination of your gene.

Multiple Cloning Site of pcDNA™5/FRT/TO

Below is the multiple cloning site for pcDNA™5/FRT/TO. Restriction sites are labeled to indicate the cleavage site. Potential stop codons are underlined. The multiple cloning site has been confirmed by sequencing and functional testing. For a map and a description of the features of pcDNA™5/FRT/TO, refer to the **Appendix, pages 9–10. The complete sequence of pcDNA™5/FRT/TO is available for downloading from www.invitrogen.com or from Technical Support (see page 12).**

```
                                                                                CMV Forward priming site
 721  AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG

                                                 TATA box                                Tetracycline operator (TetO2)
 781  GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CCCTATCAGT GATAGAGATC

      Tetracycline operator (TetO2)
 841  TCCCTATCAG TGATAGAGAT CGTCGACGAG CTCGTTTAGT GAACCGTCAG ATCGCCTGGA

 901  GACGCCATCC ACGCTGTTTT GACCTCCATA GAAGACACCG GGACCGATCC AGCCTCCGGA

                  Pme I*  Afl II  Hind III  Asp718 I  Kpn I        BamH I                          BstX I*
 961  CTCTAGCGTT TAAACTTAAG CTTGGTACCG AGCTCGGATC CACTAGTCCA GTGTGGTGGA

                     EcoR V          BstX I*  Not I       Xho I          Eco0109 I  Apa I    Pme I*
1021  ATTCTGCAGA TATCCAGCAC AGTGGCGGCC GCTCGAGTCT AGAGGGCCCG TTTAAACCCG

                        BGH Reverse priming site
1081  CTGATCAGCC TCGACTGTGC CTTCTAGTTG CCAGCCATCT
```

*Note that there are two *Pme* I sites and two *Bst*X I sites in the polylinker.

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Cloning into pcDNA™5/FRT/TO, continued

E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (*e.g.*, TOP10, DH5α™-T1^R) and select on LB agar plates containing 50 to 100 µg/ml ampicillin. Select 10–20 clones and analyze for the presence and orientation of your insert.

We recommend that you sequence your construct with the CMV Forward and BGH Reverse primers to confirm that your gene is in the correct orientation for expression and contains an ATG initiation codon and a stop codon. See the previous page for the location of the primer binding sites.

Primer	Sequence
BGH Reverse	5′-TAGAAGGCACAGTCGAGG-3′
CMV Forward	5′-CGCAAATGGGCGGTAGGCGTG-3′

For your convenience, Invitrogen offers a custom primer synthesis service. Go to www.invitrogen.com for more details.

Preparing 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_{600} = 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

Introduction

Once you have cloned your gene of interest into pcDNA™5/FRT/TO and have prepared clean plasmid preparations of your pcDNA5/FRT/TO construct and pOG44, you are ready to cotransfect the plasmids into your mammalian Flp-In™ T-REx™ host cell line to generate your stable Flp-In™ T-REx™ expression cell line. We recommend that you include the pcDNA™5/FRT/TO/CAT positive control vector and a mock transfection (negative control) to evaluate your results. General information about transfection and selection is provided below. Specific guidelines and protocols for generation of the Flp-In™ T-REx™ expression cell line can be found in the Flp-In™ T-REx™ Core Kit manual.

For detailed information about pOG44 and generation of the Flp-In™ T-REx™ host cell line, refer to the Flp-In™ T-REx™ Core Kit manual.

The Flp-In™ T-REx™-293 host cell line is available from Invitrogen to facilitate generation of your Flp-In™ T-REx™ expression cell line (see page vi for ordering information). The Flp-In™ T-REx™-293 cell line stably expresses the *lacZ-Zeocin*™ fusion gene and the Tet repressor, and contains a single integrated FRT site. If you wish to express your gene of interest in 293, you may want to use this Flp-In™ T-REx™ host cell line to establish your expression cell line. For more information, go to www.invitrogen.com or contact Technical Support (see page 12).

Flp-In™ Host Cell Lines

Several Flp-In™ host cell lines are also available from Invitrogen. Flp-In™ host cell lines stably express the *lacZ-Zeocin*™ fusion gene and contain a single integrated FRT site, but do not express the Tet repressor. By simply transfecting the pcDNA™6/TR plasmid into a Flp-In™ host cell line, a Flp-In™ T-REx™ host cell line can be generated. For more information about the Flp-In™ cell lines and pcDNA™6/TR go to www.invitrogen.com or contact Technical Support (see page 12).

Note: It is possible to cotransfect pcDNA™5/FRT/TO and pOG44 into a Flp-In™ host cell line to generate an expression cell line. In this case, the $TetO_2$ sequences in the hybrid CMV/$TetO_2$ promoter of pcDNA™5/FRT/TO are inert and the CMV/$TetO_2$ promoter functions to allow constitutive expression of your gene of interest at levels similar to the native CMV promoter.

Important

We have observed down-regulation of the viral CMV promoter and subsequent loss of gene expression when pcDNA™5/FRT-based expression constructs are introduced into Flp-In™-3T3 or Flp-In™-BHK cells. We recommend that you **DO NOT** use 3T3 or BHK cells when generating your Flp-In™ T-REx™ host cell line.

Plasmid Preparation

Plasmid DNA for transfection into eukaryotic cells must be clean and free of contamination 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 PureLink™ HQ Mini Plasmid Purification Kit (page v). Other methods of obtaining high quality plasmid DNA may be suitable.

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Transfection, continued

Positive Control

pcDNA™5/FRT/TO/CAT is provided as a positive control vector for mammalian cell transfection and expression (see page 11) and may be used to assay for recombinant protein expression levels in your Flp-In™ T-REx™ expression cell line. Cotransfection of the positive control vector and pOG44 into your Flp-In™ T-REx™ host cell line allows you to generate a stable cell line which inducibly expresses chloramphenicol acetyl transferase (CAT) at the same genomic locus as your gene of interest. If you have several different Flp-In™ T-REx™ host cell lines, you may use the pcDNA™5/FRT/TO/CAT control vector to compare protein expression levels between the various cell lines.

Assay for CAT Protein

The CAT protein expressed from the pcDNA™5/FRT/TO/CAT control plasmid is approximately 32 kDa in size. You may assay for CAT expression by ELISA assay, western blot analysis, fluorometric assay, or radioactive assay (Ausubel *et al.*, 1994; Neumann *et al.*, 1987). The Anti-CAT Antiserum (Cat. no. R902-25) is available from Invitrogen for detection of CAT protein by western blot analysis.

Hygromycin B

The pcDNA™5/FRT/TO vector contains the hygromycin resistance gene (Gritz and Davies, 1983) for selection of stable transfectants with the antibiotic, hygromycin B (Palmer *et al.*, 1987). When added to cultured mammalian cells, hygromycin B acts as an aminocyclitol to inhibit protein synthesis. Hygromycin B liquid is available separately from Invitrogen (Cat. no. 10687-010). For instructions to handle and store hygromycin B, refer to the Flp-In™ T-REx™ Core Kit manual.

Determination of Hygromycin Sensitivity

Before generating a stable cell line that inducibly expresses your protein of interest (Flp-In™ T-REx™ expression cell line), we recommend that you generate a kill curve to determine the minimum concentration of hygromycin required to kill your untransfected Flp-In™ T-REx™ host cell line. Generally, concentrations between 10 and 400 µg/ml hygromycin are required for selection of most mammalian cell lines. General guidelines for performing a kill curve are provided in the Flp-In™ T-REx™ Core Kit manual.

Generation of Flp-In™ T-REx™ Expression Cell Lines

To generate Flp-In™ T-REx™ expression cell lines, you will cotransfect your pcDNA™5/FRT/TO expression construct and pOG44 into the Flp-In™ T-REx™ host cell line and use hygromycin to select for stable transfectants. Refer to the Flp-In™ T-REx™ Core Kit manual for detailed guidelines and instructions for transfection and selection.

Induction of Gene Expression

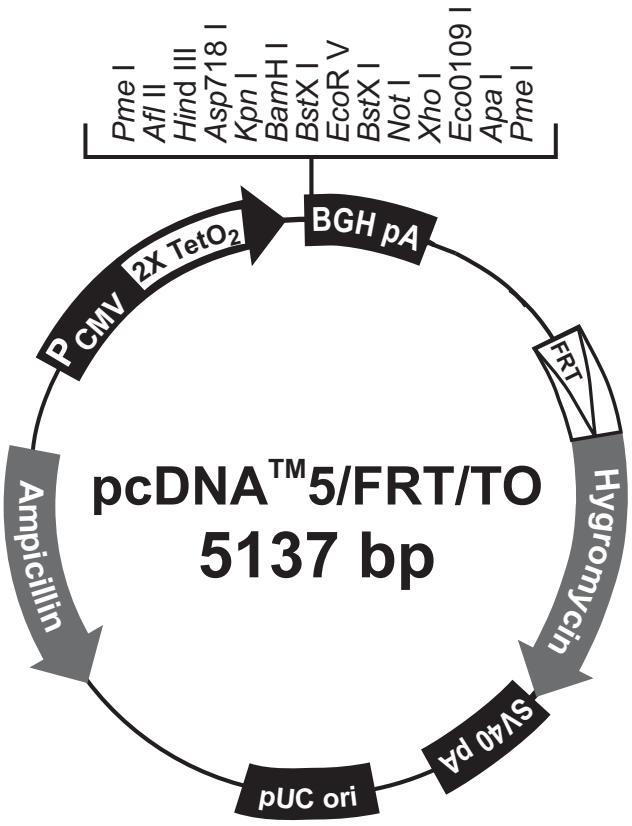
Once you have generated a Flp-In™ T-REx™ expression cell line, you will use tetracycline to induce expression of the gene of interest. We generally use 1 µg/ml tetracycline and treat cells for 24 hours to induce expression. Expression conditions may vary depending on the nature of your gene of interest and the cell line, therefore, we recommend that you perform dose response and/or time course experiments to optimize expression conditions for your protein of interest. For protocols and guidelines to prepare tetracycline and induce expression of your protein of interest, refer to the Flp-In™ T-REx™ Core Kit manual.

Appendix

pcDNA™5/FRT/TO Vector

Map of pcDNA™5/FRT/TO

The figure below summarizes the features of the pcDNA™5/FRT/TO vector. Note that the hygromycin resistance gene lacks a promoter and its native ATG start codon. Transfection of the pcDNA™5/FRT/TO plasmid alone into mammalian cells will **not** confer hygromycin resistance to the cells. **The complete nucleotide sequence for pcDNA™5/FRT/TO is available for downloading from www.invitrogen.com or by contacting Technical Support (see page 12).**



Comments for pcDNA™5/FRT/TO 5137 nucleotides

CMV promoter: bases 232-958
TATA box: bases 804-810
Tetracycline operator (2X $TetO_2$) 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 signal: bases 1095-1319
FRT site: bases 1603-1650
Hygromycin resistance gene (no ATG): bases 1658-2678
SV40 early polyadenylation signal: bases 2810-2940
pUC origin: bases 3323-3996 (complementary strand)
bla promoter: bases 5002-5100 (complementary strand)
Ampicillin (*bla*) resistance gene: bases 4141-5001 (complementary strand)

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pcDNA™5/FRT/TO Vector, continued

Features of pcDNA™5/FRT/TO

pcDNA™5/FRT/TO is a 5137 bp vector that inducibly expresses your gene of interest under the control of a hybrid CMV/TetO_2 promoter. The table below describes the relevant features of pcDNA™5/FRT/TO. All features have been functionally tested.

Feature	Benefit
Human cytomegalovirus (CMV) immediate early promoter	Permits high-level expression of your gene of interest (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987)
CMV Forward priming site	Allows sequencing in the sense orientation
Tetracycline operator 2 (O_2) sequences	Two tandem 19 nucleotide repeats which serve as binding sites for tet repressor homodimers (Hillen and Berens, 1994; Hillen *et al.*, 1983)
Multiple cloning site	Allows insertion of your gene of interest
BGH Reverse priming site	Permits sequencing of the non-coding strand
Bovine growth hormone (BGH) polyadenylation signal	Permits efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)
Flp Recombination Target (FRT) site	Encodes a 34 bp (+14 bp of non-essential) sequence that serves as the binding and cleavage site for Flp recombinase (Gronostajski and Sadowski, 1985; Jayaram, 1985; Senecoff *et al.*, 1985)
Hygromycin resistance gene (no ATG)	Permits selection of stable transfectants in mammalian cells (Gritz and Davies, 1983) when brought in frame with a promoter and an ATG initiation codon through Flp recombinase-mediated recombination via the FRT site
SV40 early polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA
pUC origin	Allows high-copy number replication and growth in *E. coli*
bla promoter	Allows expression of the ampicillin (*bla*) resistance gene
Ampicillin (*bla*) resistance gene (β-lactamase)	Permits selection of transformants in *E. coli*

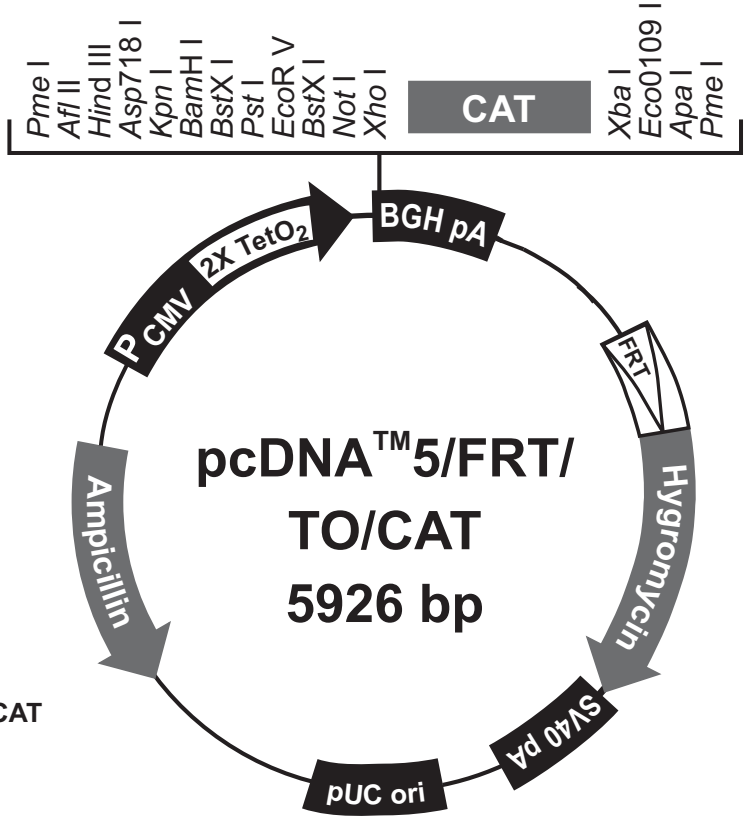
pcDNA™5/FRT/TO/CAT Vector

Description

pcDNA™5/FRT/TO/CAT is a 5926 bp control vector containing the gene for chloramphenicol acetyl transferase (CAT). This vector was constructed by ligating a 0.7 kb *Xho* I-*Apa* I fragment containing the *CAT* gene into the *Xho* I-*Apa* I site of pcDNA™5/FRT/TO. The CAT protein expressed from pcDNA™5/FRT/TO/CAT is about 32 kDa in size.

Map of pcDNA™5/FRT/CAT

The figure below summarizes the features of the pcDNA™5/FRT/TO/CAT vector. **The complete nucleotide sequence for pcDNA™5/FRT/TO/CAT is available for downloading from www.invitrogen.com or from Technical Support (see the next page).**



Comments for pcDNA™5/FRT/TO/CAT
5926 nucleotides

CMV promoter: bases 232-958
TATA box: bases 804-810
Tetracycline operator (2X $TetO_2$) sequences: bases 820-859
CMV forward priming site: bases 769-789
CAT ORF: bases 1093-1752
BGH reverse priming site: bases 1878-1895
BGH polyadenylation signal: bases 1884-2108
FRT site: bases 2392-2439
Hygromycin resistance gene (no ATG): bases 2447-3467
SV40 early polyadenylation signal: bases 3599-3729
pUC origin: bases 4112-4785 (complementary strand)
bla promoter: bases 5791-5889 (complementary strand)
Ampicillin (*bla*) resistance gene: bases 4930-5790 (complementary strand)

Technical Support

Web Resources

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

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E-mail: eurotech@invitrogen.com

MSDS

MSDSs (Material Safety Data Sheets) are available on our web site at www.invitrogen.com/msds.

Certificate of Analysis

The Certificate of Analysis (CofA) provides detailed quality control information for each product and is searchable by product lot number, which is printed on each box. CofAs are .available on our website at www.invitrogen.com/support.

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References

Andersson, S., Davis, D. L., Dahlbäck, H., Jörnvall, H., and Russell, D. W. (1989). Cloning, Structure, and Expression of the Mitochondrial Cytochrome P-450 Sterol 26-Hydroxylase, a Bile Acid Biosynthetic Enzyme. J. Biol. Chem. *264*, 8222-8229.

Andrews, B. J., Proteau, G. A., Beatty, L. G., and Sadowski, P. D. (1985). The FLP Recombinase of the 2 Micron Circle DNA of Yeast: Interaction with its Target Sequences. Cell *40*, 795-803.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994). Current Protocols in Molecular Biology (New York: Greene Publishing Associates and Wiley-Interscience).

Boshart, M., Weber, F., Jahn, G., Dorsch-Häsler, K., Fleckenstein, B., and Schaffner, W. (1985). A Very Strong Enhancer is Located Upstream of an Immediate Early Gene of Human Cytomegalovirus. Cell *41*, 521-530.

Craig, N. L. (1988). The Mechanism of Conservative Site-Specific Recombination. Ann. Rev. Genet. *22*, 77-105.

Goodwin, E. C., and Rottman, F. M. (1992). The 3´-Flanking Sequence of the Bovine Growth Hormone Gene Contains Novel Elements Required for Efficient and Accurate Polyadenylation. J. Biol. Chem. *267*, 16330-16334.

Gritz, L., and Davies, J. (1983). Plasmid-Encoded Hygromycin-B Resistance: The Sequence of Hygromycin-B-Phosphotransferase Gene and its Expression in *E. coli* and *S. cerevisiae*. Gene *25*, 179-188.

Gronostajski, R. M., and Sadowski, P. D. (1985). Determination of DNA Sequences Essential for FLP-mediated Recombination by a Novel Method. J. Biol. Chem. *260*, 12320-12327.

Hillen, W., and Berens, C. (1994). Mechanisms Underlying Expression of Tn*10* Encoded Tetracycline Resistance. Annu. Rev. Microbiol. *48*, 345-369.

Hillen, W., Gatz, C., Altschmied, L., Schollmeier, K., and Meier, I. (1983). Control of Expression of the Tn*10*-encoded Tetracycline Resistance Genes: Equilibrium and Kinetic Investigations of the Regulatory Reactions. J. Mol. Biol. *169*, 707-721.

Jayaram, M. (1985). Two-micrometer Circle Site-specific Recombination: The Minimal Substrate and the Possible Role of Flanking Sequences. Proc. Natl. Acad. Sci. USA *82*, 5875-5879.

Kozak, M. (1987). An Analysis of 5´-Noncoding Sequences from 699 Vertebrate Messenger RNAs. Nuc. Acids Res. *15*, 8125-8148.

Kozak, M. (1991). An Analysis of Vertebrate mRNA Sequences: Intimations of Translational Control. J. Cell Biol. *115*, 887-903.

Kozak, M. (1990). Downstream Secondary Structure Facilitates Recognition of Initiator Codons by Eukaryotic Ribosomes. Proc. Natl. Acad. Sci. USA *87*, 8301-8305.

Nelson, J. A., Reynolds-Kohler, C., and Smith, B. A. (1987). Negative and Positive Regulation by a Short Segment in the 5´-Flanking Region of the Human Cytomegalovirus Major Immediate-Early Gene. Mol. Cell. Biol. *7*, 4125-4129.

Neumann, J. R., Morency, C. A., and Russian, K. O. (1987). A Novel Rapid Assay for Chloramphenicol Acetyltransferase Gene Expression. BioTechniques *5*, 444-447.

Palmer, T. D., Hock, R. A., Osborne, W. R. A., and Miller, A. D. (1987). Efficient Retrovirus-Mediated Transfer and Expression of a Human Adenosine Deaminase Gene in Diploid Skin Fibroblasts from an Adenosine-Deficient Human. Proc. Natl. Acad. Sci. U.S.A. *84*, 1055-1059.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Second Edition (Plainview, New York: Cold Spring Harbor Laboratory Press).

Sauer, B. (1994). Site-Specific Recombination: Developments and Applications. Curr. Opin. Biotechnol. *5*, 521-527.

Senecoff, J. F., Bruckner, R. C., and Cox, M. M. (1985). The FLP Recombinase of the Yeast 2-micron Plasmid: Characterization of its Recombination Site. Proc. Natl. Acad. Sci. USA *82*, 7270-7274.

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