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# pcDNA™ 3.4-TOPO® TA Cloning Kit

| Cat. no. | Amount | Storage |
|---|---|---|
| A14697 | 20 reactions | See below |

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## Kit Contents and Storage

The pcDNA™ 3.4–TOPO® TA Cloning Kit is shipped on dry ice. Each kit contains two boxes; upon receipt, store their contents as detailed below.

| | Item | Amount | Storage* |
|---|---|---|---|
| Box 1 | pcDNA™ 3.4-TOPO® vector (5–10 ng/µL) | 20 µL | –20°C |
| | 10X PCR buffer | 100 µL | –20°C |
| | dNTP Mix (12.5 mM each dATP, dCTP, dGTP, dTTP) | 10 µL | –20°C |
| | Salt solution | 50 µL | –20°C |
| | Sterile water | 1 mL | RT |
| | Control PCR template (50 ng/µL) | 10 µL | –20°C |
| | Control PCR primers (100 ng/µL) | 10 µL | –20°C |
| | CMV forward sequencing primer (100 ng/µL) | 20 µL | –20°C |
| | pcDNA™ 3.4 reverse sequencing primer (2 µg/µL) | 20 µL | –20°C |
| Box 2 | One Shot® TOP10 Chemically Competent *E. coli* | 21 × 50 µL | –80°C |
| | S.O.C. medium | 6 mL | 4°C or RT |
| | pUC19 control DNA (10 pg/µL) | 50 µL | –20°C |

* For convenience, you may store the entire Box 1 at –20°C, and Box 2 at –80°C.
RT: room temperature.

## Description

The pcDNA™ 3.4-TOPO® vector allows the rapid TOPO® cloning of a PCR product containing a gene of interest downstream of the CMV promoter for high level expression of the native protein in adherent mammalian cell cultures, or for high level expression of secreted, native protein in suspension mammalian cell cultures following transient transfection.

The instructions provided below are intended for experienced users. For detailed protocols and more information on the pcDNA™ 3.4-TOPO® vector, refer to the pcDNA™ 3.4–TOPO® TA Cloning Kit manual available online at **www.lifetechnologies.com/manuals**.

## Guidelines for Designing PCR Primers

To obtain the pcDNA™ 3.4-TOPO® expression construct containing your gene of interest, your PCR primer design must include:

- a Kozak consensus sequence
- a mammalian secretion signal upstream of your gene of interest (if you wish to produce secreted protein)
- a stop codon at the end of your gene of interest

## Amplify Your Gene of Interest

1. Produce PCR products containing your gene of interest using *Taq* polymerase (see **Note** below). Use cycling parameters suitable for your primers and template and include a final extension step of 7–10 minutes to ensure 3′ adenylation.
2. Verify there is only one PCR product of the proper size in each reaction by agarose gel electrophoresis.

**Note:** We recommend using Platinum® *Taq* DNA Polymerase High Fidelity (Cat. no. 11304). You may use an enzyme mixture containing *Taq* polymerase and a proofreading polymerase; however *Taq* must be used in excess of 10:1 to ensure the presence of 3′ A-overhangs on the PCR product. If you use polymerase mixtures that do not have enough *Taq* polymerase or use a proofreading polymerase only, you can add 3′ A-overhangs after amplification (refer to the online manual for protocol).

## Perform TOPO® Cloning

1. Set up the following 6 µL TOPO® Cloning reaction for each PCR product, adding the reagents in the order shown. For optimal results, use a 0.5:1 to 2:1 molar ratio of PCR product:TOPO® vector.

| Reagent | Volume |
|---|---|
| PCR product | 0.5–4 µL |
| Salt solution | 1 µL |
| Sterile water | to 5 µL |
| pcDNA™ 3.4-TOPO® vector | 1 µL |

2. Mix gently and incubate for 5 minutes at room temperature.
3. Place tubes on ice and proceed to transformation.

## Transform One Shot® TOP10 Chemically Competent *E. coli*

1. Thaw One Shot® *E. coli* or equivalent competent cells on ice.
2. Add 2 µL of the TOPO® Cloning reaction to a vial of One Shot® TOP10 Chemically Competent *E. coli* or equivalent, and mix gently.
3. Incubate on ice for 5–30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking and immediately transfer the cells to ice.
5. Add 250 µL of room temperature S.O.C. Medium.
6. Cap the tube tightly and incubate at 37°C for 1 hour with horizontal shaking at 200 rpm.
7. Spread 10–50 µL from each transformation on a pre-warmed selective LB plate and incubate overnight.

## Analyze Positive Clones

1. Pick 10 colonies and culture them overnight in LB medium containing 100 µg/mL ampicillin.
2. Isolate plasmid DNA using your method of choice.
3. Analyze the plasmid by restriction analysis or by sequencing to confirm the presence and correct orientation of the insert.

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