

USER GUIDE

invitrogen™
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One Shot® TOP10 Competent Cells

Catalog Numbers C4040-10, C4040-03, C4040-06, C4040-50, and C4040-52

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About this guide

IMPORTANT!

Before using this product, read and understand the information in the “Safety” appendix in this document.

Changes from previous version

Revision	Date	Description
A.0	December 2013	<ul style="list-style-type: none">• Replaced Product Qualification section with Obtaining Certificates of Analysis on page 13.• Version numbering changed to alphanumeric format and reset to A in conformance with internal document control procedures

Product information

Contents

Types of products

This manual is supplied with the following products.

Product	Reactions	Cat. no.
One Shot® TOP10 Chemically Competent <i>E. coli</i>	10	C4040-10
	20	C4040-03
	40	C4040-06
One Shot® TOP10 Electrocomp™ <i>E. coli</i>	10	C4040-50
	20	C4040-52

Contents

Each product contains the following components.

Type of Cells	Number of Tubes	Volume
Chemically Competent	11 (10 rxn), 21 (20 rxn), or 42 (40 rxn)	50 µL
Electrocomp™	11 (10 rxn), 21 (20 rxn)	50 µL

All kits contain the following reagents:

- 50 µL supercoiled pUC19 plasmid (10 pg/µL in 5 mM Tris-HCl, 5 mM EDTA, pH 8) for testing efficiency
- S.O.C Medium (6 mL) for plating

Description of the system

Genotype	<i>F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara-leu)7697 galU galK rpsL (Str^R) endA1 nupG</i>
General handling	Be extremely gentle when working with competent cells. Competent cells are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Transformation should be started immediately following the thawing of the cells on ice. Mix by swirling or tapping the tube gently, not by pipetting.
IMPORTANT!	<p>One Shot® TOP10 cells do not require IPTG to induce expression from the <i>lac</i> promoter.</p> <p>If blue/white screening is required to select for transformants spread 40 µL of 40 mg/mL X-Gal in dimethylformamide on top of the agar. Let the X-Gal diffuse into the agar for approximately 1 hour.</p>

Methods

Transform chemically competent cells

Introduction

This section provides two procedures to transform One Shot® TOP10 chemically competent *E. coli*. See **Selecting a One Shot® chemical transformation procedure** below to help you choose the best procedure to use for your needs.

Selecting a One Shot® chemical transformation procedure

Two procedures are provided to transform One Shot® TOP10 chemically competent *E. coli*. Consider the following factors when choosing which procedure to use. Note that if you use the rapid chemical transformation procedure, fewer transformants will be obtained.

If you wish to...	Then use the...
maximize the number of transformants obtained	regular Chemical transformation procedure (see page 8)
use an antibiotic other than ampicillin to select for your plasmid (<i>e.g.</i> kanamycin)	
transform a plasmid containing a large insert (>1000 bp)	
obtain transformants as quickly as possible	Rapid chemical transformation procedure (see page 8)

IMPORTANT!

The rapid chemical transformation procedure is only suitable for transformations using ampicillin selection. If you will be using any other antibiotic for selection (*e.g.* kanamycin), use the regular chemical transformation procedure.

Materials needed

- 37°C shaking and non-shaking incubator
- 10-cm diameter LB agar plates with appropriate antibiotic
- Ice bucket with ice
- 42°C water bath

Before starting

- Equilibrate a water bath to 42°C.
- Warm the vial of S.O.C medium to room temperature.
- Spread X-Gal onto LB agar plates with antibiotic, if desired for blue/white selection.
- Warm the selective plates in a 37°C incubator for 30 minutes (use one plate for each transformation).

IMPORTANT! It is essential that LB plates containing 100 µg/mL ampicillin are pre-warmed if you are performing the rapid chemical transformation procedure.

continued on next page

Transform chemically competent cells, continued

Chemical transformation procedure

The instructions provided below are for general use. Specific instructions for particular applications such as Zero Blunt® PCR Cloning are provided in the manual for that kit.

1. Centrifuge the vial(s) containing the ligation reaction(s) briefly and place on ice.
2. Thaw, on ice, one 50 µL vial of One Shot® cells for each ligation/transformation.
3. Pipet 1–5 µL of each ligation reaction directly into the vial of competent cells and mix by tapping gently. **Do not mix by pipetting up and down.** The remaining ligation mixture(s) can be stored at –20°C.
4. Incubate the vial(s) on ice for 30 minutes.
5. Incubate for exactly 30 seconds in the 42°C water bath. Do not mix or shake.
6. Remove vial(s) from the 42°C bath and place them on ice.
7. Add **250 µL** of pre-warmed S.O.C medium to each vial. S.O.C is a rich medium; sterile technique must be practiced to avoid contamination.
8. Place the vial(s) in a microcentrifuge rack on its side and secure with tape to avoid loss of the vial(s). Shake the vial(s) at 37°C for exactly 1 hour at 225 rpm in a shaking incubator.
9. Spread 20–200 µL from each transformation vial on separate, labeled LB agar plates. The remaining transformation mix may be stored at 4°C and plated out the next day, if desired.
10. Invert the plate(s) and incubate at 37°C overnight.
11. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

Rapid chemical transformation procedure

An alternative procedure is provided below for rapid transformation of One Shot® chemically competent *E. coli*. This procedure is only recommended for transformations using **ampicillin** selection.

Note: It is essential that selective plates be pre-warmed prior to spreading.

1. Centrifuge the vial(s) containing the ligation reaction(s) briefly and place on ice.
2. Thaw, on ice, one 50 µL vial of One Shot® cells for each ligation/transformation.
3. Pipet 1–5 µL of each ligation reaction directly into the vial of competent cells and mix by tapping gently. **Do not mix by pipetting up and down.** The remaining ligation mixture(s) can be stored at –20°C.
4. Incubate the vial(s) on ice for 5 minutes.
5. Spread 50 µL of cells on a pre-warmed, labeled LB agar plate containing 100 µg/mL ampicillin and incubate at 37°C overnight.
6. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

if efficiency is low, try 1.5 - 2.0 kV
per tech support

Transform Electrocomp™ Cells

Materials needed

- 37°C shaking and non-shaking incubator
- 10-cm diameter LB agar plates with appropriate antibiotic
- Ice bucket with ice
- Electroporator
- Cuvettes (0.1- or 0.2-cm, see **Note**)
- 15-mL snap-cap tubes (one for each transformation)

Note

One Shot® Electrocomp™ cells are supplied in 50 µL single-use aliquots. Please refer to the user manual included with your electroporator for cuvette size and reaction volume. You may dispose of any unused cells.

Preparation

For each transformation, you will need one vial of competent cells and at least one selective plate.

- Thaw the vial of S.O.C medium and bring to room temperature.
- Spread X-Gal onto LB agar plates with antibiotic, if desired.
- Warm selective plates at 37°C for 30 minutes.
- Place cuvettes on ice.
- Thaw **on ice** 1 vial of One Shot® Electrocomp™ cells for each transformation.

Electroporation procedure

The instructions provided below are for general use. Specific instructions for particular applications such as TOPO® XL PCR Cloning are provided in the manual for that kit.

Note: For transformation of large plasmids, electroporation is preferred over chemical transformation because not only is the transformation efficiency higher, it is less biased against large recombinant plasmids.

IMPORTANT! To avoid arcing, use **only** Electrocomp™ cells for electroporation.

1. Set up your electroporator for bacterial transformation. Follow the manufacturer's instructions.
2. Add 1–2 µL of each ligation reaction to the volume of cells recommended by the manufacturer (may be less than 50 µL). Mix gently with pipette tip. **Do not mix by pipetting up and down.**
3. Transfer the cells to the chilled electroporation cuvette on ice.
4. Electroporate the cells as per the manufacturer's recommended protocol.
5. Quickly add 250 µL room temperature S.O.C medium and mix gently.
6. Transfer the solution to a 15-mL snap-cap tube (i.e. Falcon) and shake for at least 1 hour at 37 °C to allow expression of the antibiotic resistance gene.
7. Spread 10–150 µL from each transformation on a prewarmed LB plate containing the appropriate antibiotic. The remaining transformation mix may be stored at 4°C and plated out the next day, if desired.
8. Incubate the plates overnight at 37°C.
9. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

Transformation control

Test the efficiency

We recommend that you test the efficiency of the competent cells contained in the One Shot® Kit. This can be accomplished by using the supercoiled pUC19 plasmid supplied with the kit as described below.

1. Prepare LB agar plates containing 100 µg/mL ampicillin
2. Transform 1 µL (10 pg) into 50 µL of competent cells according to the transformation protocol appropriate for the type of cells.
3. Plate the control transformation as follows:

Competent Cells	pUC19 (pg)	Volume to Plate
Electrocomp™	10	20 µL (1:10 dilution)*
Chemically Competent	10	10 µL + 20 µL SOC

*Just before plating the Electrocomp™ transformation mix, dilute 10 µL of the transformation mix with 90 µL of S.O.C medium.

4. Incubate overnight at 37°C and count colonies. Calculate transformation efficiency using the formula below.

Calculation

Calculate the transformation efficiency as transformants per 1 µg of plasmid DNA.

For chemically competent cells, use the formula below to calculate transformation efficiency:

$$\frac{\text{\# of colonies}}{10 \text{ pg transformed DNA}} \times \frac{10^6 \text{ pg}}{\mu\text{g}} \times \frac{300 \mu\text{L total transformation volume}}{X \mu\text{L plated}} = \frac{\text{\# transformants}}{\mu\text{g plasmid DNA}}$$

For Electrocomp™ cells, use the formula below to calculate transformation efficiency:

$$\frac{\text{\# of colonies}}{10 \text{ pg transformed DNA}} \times \frac{10^6 \text{ pg}}{\mu\text{g}} \times \frac{\text{Total transformation volume}^*}{X \mu\text{L plated}} \times 10 = \frac{\text{\# transformants}}{\mu\text{g plasmid DNA}}$$

*Volume dependent on the volume of cells used and the amount of S.O.C. added.

Expected transformation efficiency:

Cells	Transformation Efficiency
Chemically competent	$\geq 1 \times 10^9$ cfu/µg supercoiled plasmid
Electrocomp™	$\geq 1 \times 10^9$ cfu/µg supercoiled plasmid

Appendix A: Safety

Chemical safety

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, and 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

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. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Documentation and Support

Obtaining support

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining SDSs

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/sds**.

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Technical Support

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 - Submit a question directly to Technical Support (**techsupport@lifetech.com**)
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Notes

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