One Shot® TOP10 Competent Cells

Catalog nos. C4040-10, C4040-03, C4040-06, C4040-50, and C4040-52

Version M
6 April 2004
28-0126

www.invitrogen.com
tech_service@invitrogen.com

Important Information

Introduction

This manual is supplied with the following kits:

Kit	Reactions	Catalog no.
One Shot® TOP10 Chemically Competent *E. coli*	10	C4040-10
	20	C4040-03
	40	C4040-06
One Shot® TOP10 Electrocomp™ *E. coli*	10	C4040-50
	20	C4040-52

Contents

Each kit contains the following:

Type of Cells	Number of Tubes	Volume per Tube
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

Genotype

F⁻ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G

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

One Shot® TOP10cells **do not require** IPTG to induce expression from the *lac* promoter.

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.

continued on next page

Product Qualification

All competent cells are qualified as follows:

- Cells are tested for transformation efficiency using the control plasmid included in the kit. Transformed cultures are plated on LB plates containing 100 μg/ml ampicillin and the transformation efficiency is calculated. Test transformations are performed in duplicate. Transformation efficiency should be ~1 x 10^9 cfu/μg DNA for chemically competent cells and >1 x 10^9 for electrocompetent cells.
- To verify the absence of phage contamination, 0.5-1 ml of competent cells are added to LB top agar and poured onto LB plates. After overnight incubation, no plaques should be detected.
- Untransformed cells are plated on LB plates 100 μg/ml ampicillin, 25 μg/ml streptomycin, 50 μg/ml kanamycin, or 15 μg/ml chloramphenicol to verify the absence of antibiotic-resistant contamination.

Methods

Transforming 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 4)
use an antibiotic other than ampicillin to select for your plasmid (*e.g.* kanamycin)	
transform a plasmid containing a large insert (>1000 bp)	
obtain transformants as quickly as possible	rapid chemical transformation procedure (see page 4)

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 Supplied by the User

You will need the following items for transformation:

- 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

Transforming 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 to 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 µl to 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.

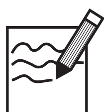
1. Centrifuge the vial(s) containing the ligation reaction(s) briefly and place on ice.
2. Thaw, on ice, one 50 µl vial of One Shot® cells for each ligation/transformation.
3. Pipet 1 to 5 µl of each ligation reaction directly into the vial of competent cells and mix by tapping gently. **Do not mix by 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.

Transforming Electrocomp™ Cells

Materials Supplied by the User

You will need the following items for transformation:

- 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.

continued on next page

Transforming Electrocomp™ Cells, continued

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 to 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

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:

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

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

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

*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 x 10^9 cfu/μg supercoiled plasmid
Electrocomp™	$\geq$1 x 10^9 cfu/μg supercoiled plasmid

Appendix

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact Us

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

Corporate Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008
USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail: tech_service@invitrogen.com

Japanese Headquarters:
Invitrogen Japan K.K.
Nihonbashi Hama-Cho Park
Bldg. 4F
2-35-4, Hama-Cho, Nihonbashi
Tel: 81 3 3663 7972
Fax: 81 3 3663 8242
E-mail: jpinfo@invitrogen.com

European Headquarters:
Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

MSDS Requests

To request an MSDS, visit our Web site at www.invitrogen.com. On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

continued on next page

Technical Service, continued

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, please contact our Technical Service Representatives.

Invitrogen warrants that all of its products will perform according to the specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order.

Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Service Representatives.

Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

©1997-2004 Invitrogen Corporation. All rights reserved.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.