One Shot™ TOP10 Chemically Competent *E. coli*

Catalog Number C4040-10 (10 reactions), C4040-03 (20 reactions), and C4040-06 (40 reactions)

Doc. Part No. C4040.pps **Pub. No.** MAN0001491 **Rev.** A.0

WARNING! This product contains irritants and may be harmful if swallowed. Review the Material Safety Data Sheet before handling. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

TOP10 *E. coli* are provided at a transformation efficiency of 1×10^9 cfu/µg supercoiled DNA and are ideal for high-efficiency cloning and plasmid propagation. They allow stable replication of high-copy number plasmids. The genotype of TOP10 Cells is similar to the DH10B™ strain.

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 λ-

Contents and storage

Component	Quantity	Storage conditions
TOP10 Chemically Competent *E. coli* Cells	21 × 50 µL	Store at –80°C
pUC19 Control DNA (10 pg/µL)	50 µL	
S.O.C. Medium	6 mL	

Important guidelines

Follow these guidelines when using Invitrogen™ One Shot™ TOP10 Chemically Competent *E. coli*.

- Handle competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Thaw One Shot™ competent cells on ice, and transform cells immediately following thawing. After adding DNA, mix by swirling or tapping the tube gently. *Do not mix cells by pipetting.*
- One Shot™ TOP10 cells do not require IPTG to induce expression from the *lac* promoter. If blue/white screening is required to select for transformants, make sure that selective plates contain 50 µg/mL X–gal.

Before you begin

Perform the following before starting the transformation procedure:

1. Equilibrate a water bath to 42°C.
2. Warm the vial of S.O.C. Medium (supplied with the kit) and LB Medium (if needed) to room temperature.
3. Spread X-gal onto LB agar plates containing antibiotic, if desired.
4. Warm the selective plates in a 37°C incubator for 30 minutes (use 1 or 2 plates for each transformation). If you are including the pUC19 control, make sure that you have one LB agar plate containing 100 µg/mL ampicillin.

Methods

Calculate transformation efficiency

Use the following formula to calculate the transformation efficiency as transformants (in cfu) per µg of plasmid DNA. Remember that the total volume of the transformation mixture is 300 µL.

Transformation efficiency (# transformants/µg DNA) =

$$\frac{\text{\# of colonies}}{\text{10 pg pUC19 DNA}} \times \frac{10^6 \text{ pg}}{\mu\text{g}} \times \frac{\text{300 µL total volume}}{\text{X µL plated}} \times \text{dilution factor}$$

If transformation of 10 pg of pUC19 DNA yields 100 colonies when 30 µL of a 1:10 dilution is plated, then the transformation efficiency is:

$$\frac{\text{100 colonies}}{\text{10 pg DNA}} \times \frac{10^6 \text{ pg}}{\mu\text{g}} \times \frac{\text{300 µL total volume}}{\text{30 µL plated}} \times 10 = 1 \times 10^9 \text{ cfu/µg}$$

Transform competent cells

Use this procedure to transform One Shot™ TOP10 chemically competent *E. coli*. We recommend including the pUC19 control plasmid DNA supplied with the kit (10 pg/µL in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8) in your transformation experiment to verify the efficiency of the competent cells. *Do not* use these cells for electroporation.

Note: For a rapid transformation procedure, see "Transform competent cells (rapid transformation procedure)" on page 4.

1. Thaw, on ice, one vial of One Shot™ TOP10 chemically competent cells for each transformation.
2. Add 1 to 5 µL of the DNA (10 pg to 100 ng) into a vial of One Shot™ cells and mix gently. *Do not mix by pipetting up and down.* For the pUC19 control, add 10 pg (1 µL) of DNA into a separate vial of One Shot™ cells and mix gently.
3. Incubate the vial(s) on ice for 30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking.
5. Remove the vial(s) from the 42°C bath and place them on ice for 2 minutes.
6. Aseptically add 250 µL of pre-warmed S.O.C. Medium to each vial.
7. Cap the vial(s) tightly and shake horizontally at 37°C for 1 hour at 225 rpm in a shaking incubator.
8. Spread 20–200 µL from each transformation on a pre-warmed selective plate and incubate overnight at 37°C. We recommend that you plate two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, dilute the transformation mix 1:10 into LB Medium (e.g. remove 100 µL of the transformation mix and add to 900 µL of LB Medium) and plate 25-100 µL.
9. Store the remaining transformation mix at 4°C. Additional cells may be plated out the next day, if desired.
10. Invert the selective plate(s) and incubate at 37°C overnight.
11. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

Transform competent cells (rapid transformation procedure)

This procedure is only recommended for transformations utilizing ampicillin selection. It is essential to pre-warm selective plates prior to spreading the transformed cells.

1. Centrifuge the ligation reactions briefly and place on ice.
2. Thaw, on ice, one 50 µL vial of One Shot™ cells for each transformation.
3. Pipet 1–5 µL of each ligation reaction into the vial of competent cells and mix by tapping gently. *Do not mix by pipetting up and down.* Store remaining ligation reactions at –20°C.
4. Incubate the cells on ice for 5 minutes.
5. Heat-shock the cells for 30 seconds at 42°C without shaking.
6. Remove the vial(s) from the 42°C bath and place them on ice for 2 minutes.
7. Immediately spread 50 µL of transformed cells on a pre-warmed selective plate containing 100 µg/mL ampicillin.
8. Incubate at 37°C overnight.
9. Select colonies and analyze by plasmid isolation/restriction, PCR, or sequencing.

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 found on Life Technologies' website at **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**.

Corporate entity: Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288

For support visit **thermofisher.com/support** or email **techsupport@lifetech.com**
thermofisher.com
30 October 2015

ThermoFisher SCIENTIFIC