invitrogen™

One Shot® TOP10 Electrocomp™ *E. coli*

Cat. Nos. C4040-50 (10 reactions); C4040-52 (20 reactions)

Store at -80°C

Caution

This product contains irritants and may be harmful if swallowed. Review the Material Safety Data Sheet before handling.

Description

TOP10 *E. coli* are provided at a transformation efficiency of 1 x 10^{10} 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.

Components Supplied	Amount
TOP10 Electrocomp™ *E. coli* Cells	21 x 50 µl
pUC19 Control DNA (10 pg/µl)	50 µl
S.O.C. Medium	6 ml

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-

General Guidelines

Follow these guidelines when using One Shot® TOP10 Electrocomp™ *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.
- 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.

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Transforming Competent Cells

Perform the following before starting the transformation procedure:

- Warm the vial of S.O.C. Medium (supplied with the kit) and LB Medium (if needed) to room temperature.
- Spread X-gal onto LB agar plates containing antibiotic, if desired.
- 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.
- Place cuvettes on ice and set up your electroporator for bacterial transformation as per the manufacturer's instructions.
- One 15 ml snap-cap tube per transformation

Transformation Procedure

Use this procedure to transform One Shot® TOP10 Electrocomp™ *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 chemically competent transformation.

1. Thaw, on ice, one vial of One Shot® TOP10 Electrocomp™ cells for each transformation.
2. Add 1-2 µl of the DNA (10 pg to 100 ng) into a vial of One Shot® cells and mix gently. For the pUC19 control, add 10 pg (1 µl) of DNA into a separate vial of One Shot® cells and mix gently.
3. Transfer the cells to the chilled electroporation cuvette on ice.
4. Electroporate the cells as per the manufacturer's recommended protocol.
5. Aseptically add 250 µl of pre-warmed S.O.C. Medium to each vial.
6. Transfer the solution to a 15 ml snap-cap tube 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 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 1:50 into LB Medium (*e.g.* remove 20 µl of the transformation mix and add to 980 µl of LB Medium) and plate 20-100 µl.
8. Store the remaining transformation mix at +4°C. Additional cells may be plated out the next day, if desired.
9. Invert the selective plate(s) and incubate at 37°C overnight.
10. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

Calculating 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) =

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

For example, if transformation of 10 pg of pUC19 DNA yields 100 colonies when 30 µl of a 1:50 dilution is plated, then the transformation efficiency is:

$$\frac{\text{100 colonies}}{\text{10 pg DNA}} \times \frac{10^6 \text{ pg}}{\mu\text{g}} \times \frac{\text{300 µl total vol.}}{\text{30 µl plated}} \times 50 = 5 \times 10^9$$

Quality Control

Each lot of TOP10 competent cells is tested for transformation efficiency using the control plasmid included in the kit and the protocol on page 2. Test transformations are performed on 3 to 20 vials per lot, depending on batch size. Transformed cultures are plated on LB plates containing 100 µg/ml ampicillin and incubated overnight. Transformation efficiency should be ~1 x 10^{10} cfu/µg plasmid DNA. In addition, untransformed cells are tested for the appropriate antibiotic sensitivity and the absence of phage contamination.