

Electrocomp™ Kits

Catalog Numbers C66455, C66411, C66511

Doc. Part No. 280078 Pub. No. MAN0000621 Rev. B.0

 **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Contents and storage

Contents	Cat. No. C66455	Cat. No. C66411	Cat. No. C66511	Storage
TOP10 Electrocomp™ <i>E. coli</i>	5 × 100 µL	10 × 100 µL	—	–80°C
TOP10F' Electrocomp™ <i>E. coli</i>	—	—	10 × 100 µL	
pUC19 vector, supercoiled (10 pg/ µL)	50 µL (in 10 mM Tris-HCl, 1 mM EDTA)			
SOC medium	15 mL			Room temperature

Genotype

TOP10: F- *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80*lacZ*Δ*M15* Δ*lacX74* *recA1* *araD139* Δ(*ara-leu*)7697 *galU* *galK* *rpsL* *endA1* *nupG*

TOP10F': F'(*lacIqTn10*(TetR)) *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80*lacZ*Δ*M15* Δ*lacX74* *recA1* *araD139* Δ(*ara-leu*)7697 *galU* *galK* *rpsL* *endA1* *nupG*

Materials required but not provided

- Electroporation device
- Electroporation cuvette
- Sterile microcentrifuge tubes
- Ice bucket for chilling cuvettes, cells, and DNA
- Sterile 15-mL tubes

Guidelines for electroporation

- For small scale electroporation (< 100 ng of DNA), use 20–40 µL of Electrocomp™ cells for a 0.1-cm cuvette, or 40–80 µL of Electrocomp™ cells for a 0.2-cm cuvettes.
- For large scale or library scale electroporation, use 160 µL of Electrocomp™ cells.
- Keep cells and cuvettes cold until after the electric pulse is applied.
- Add SOC to the cells immediately after the pulse is applied to ensure successful electroporation.

Prepare material for electroporation

- Pre-chill electroporation cuvettes on ice or at –20°C.
- Thaw SOC medium, and keep at room temperature.
Store at room temperature after initial thawing.
- Place 1–5 µL of DNA in a sterile microcentrifuge tube and chill it on ice. For the pUC19 control DNA, use 1 µL (10 pg).
Caution: Before electroporation, precipitate or desalt the DNA if in ligation or restriction buffer, then resuspend the DNA in water (do not use TE buffer) to minimize the ionic strength and prevent arcing during the pulse. Arcing can damage the machine, inhibit transformation, and cause cell death.

Enter instrument settings

Set up the instrument for bacterial electroporation according to manufacturer instructions. See the following table for settings of selected electroporation units. If using a unit not listed in the table, use the manufacturer recommended settings, though it may be necessary to modify the settings for optimal transformation efficiency.

Electroporation unit	Capacitance	Resistance	Voltage
Bio-Rad™ Gene Pulser™ II	25 µF	200 Ω	2.5 kV
BTX™ ECM™ 630	50 µF	150 Ω	1.5 kV

Prepare cells

1. Gently thaw the cells on ice. Use the cells immediately, do not leave them on ice for an extended period of time.
Unused cells can be re-frozen for later use, but will suffer significant loss of efficiency.
2. Transfer the desired volume of cells to the pre-chilled microcentrifuge tube containing the DNA. The volume of DNA added should not exceed 5% of the total cell/DNA mixture.
3. Mix the cells gently. Do not mix by pipetting up and down.
4. Incubate the cells on ice for 1 minute.

Transform cells by electroporation

1. Transfer the cell/DNA mixture to a chilled cuvette and gently shake them to the bottom of the cuvette.
Examine the cuvette from both sides to ensure the cells completely fill the bottom of the chamber without any air bubbles. Do this as quickly as possible, being careful not to warm up the cuvette and cells. Remove the condensation from the outside surfaces of the cuvette with a tissue.
2. Electroporate your sample using the entered settings.
3. Immediately add 480 µL (for small-scale) or 960 µL (for large scale) of SOC medium to the cells.
4. Transfer the suspension to a 15-mL tube. Incubate the tube at 37°C in a rotary shaking incubator at 225 rpm for 1 hour to allow expression of the antibiotic resistance.
5. Plate the cells on pre-warmed selective plates, then incubate overnight at 37°C. Plate at least two different volumes to ensure that at least one plate has well-spaced colonies.

Note: For the pUC19 control DNA, prepare a 1:10 dilution in SOC medium and plate 25 µL and 100 µL of cells on LB agar containing 100 µg/mL of ampicillin.

Limited product warranty

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