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Electrocomp™ Kits

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Contents

- 5, 10, or 30 × 100 µL electrocompetent *E. coli*
- 10 pg/µL pUC19 supercoiled vector in 10 mM Tris-HCl, 1 mM EDTA
- SOC medium, 15 mL

Genotypes

TOP10F´: F'{*lac*I^q*Tn*10(Tet^R)} *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 *end*A1 *nup*G

TOP10: F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 ΔlacX74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G

Prepare for electroporation

You must keep cells and cuvettes cold until after the pulse is applied for high efficiency electroporation. After the pulse is applied, you must immediately add SOC to the cells to ensure good results. Use the following steps to prepare for electroporation.

1. Pre-chill 0.1-cm cuvettes on ice or at –20°C.
2. Set up the electroporation device for bacterial electroporation following the directions provided by the manufacturer. See the following table of settings for selected electroporators. If you are using an electroporator not listed in the table, you may need to vary your settings to achieve optimal transformation efficiency.

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

3. Prepare an ice bucket for the cells and the DNA.
4. Thaw the SOC medium to add to the cells after the pulse. Keep the SOC at room temperature.
5. Prepare sterile 15-mL tubes for use after the pulse has been delivered

Electroporation

6. 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: Resuspend the DNA in water (rather than TE buffer) to keep the ionic strength to a minimum. Otherwise, arcing might occur during the pulse; arcing can damage the machine and results in no transformation and cell death. Precipitate or desalt DNA in ligation or restriction buffer before electroporation.
7. Gently thaw the cells on ice. Use these cells immediately; do not leave them on ice for an extended period of time. You may re-freeze unused cells for later use, but they will suffer a significant loss of efficiency.
8. Transfer the desired amount of cells to the pre-chilled microcentrifuge tube containing the DNA. We recommend using 20–40 µL (40–80 µL if using 0.2 cm cuvettes) for a small-scale electroporation (< 100 ng of DNA), and 160 µL for a large-scale or library electroporation. The volume of DNA added should not exceed 5% of the total cell/DNA mixture. Mix the cells gently. *Do not mix by pipetting up and down.* Leave the cells on ice for 1 minute.
9. After 1 minute, transfer the cells to a chilled cuvette and gently shake them to the bottom of the cuvette. Check the cuvette by looking at the contents from both sides to make sure the cells make contact all the way across 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.
10. Electroporate your sample using the settings listed or using the manufacturer's recommended settings.
11. Immediately add 480 µL (for small-scale) or 960 µL (for large-scale) of SOC medium to the cells.
12. 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.
13. Plate the cells on prewarmed selective plates and incubate overnight at 37ºC. We recommend plating 2 different volumes to ensure that at least 1 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 with 100 µg/mL of ampicillin.

Safety Data Sheets (SDSs)

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

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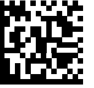

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