

Custom Electrocompetent Cells

IMPORTANT!
-80°C Storage Required
Immediately Upon Receipt

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Advanced Products for Molecular Biology

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Custom Electrocompetent Cells

Components & Storage Conditions

The cells are shipped on dry ice in one container, along with Recovery Medium and supercoiled control pUC19 DNA at 10 pg/μl.

Component	Storage
Custom Competent Cells*	-80°C
Recovery Medium**	-80°C to 20°C
Supercoiled pUC19 DNA (10pg/μl)	-80°C to 20°C

*Competent Cells require immediate storage at **-80° C**.

**Additional Recovery Medium is available for purchase (cat. no. 80026-1 (8 x 12ml))

Preparation for Transformation

Quality testing of electrocompetent cells is performed using 25 μl of competent cells per transformation in a 0.1 cm gap cuvette. Optimal settings for electroporation are listed in the table below. Typical time constants are 3.5 to 4.5 msec.

Optimal Setting	Alternate Settings (~ 20-50% lower efficiencies)
0.1 cm cuvette 10 μF 600 Ohms 1800 Volts	0.1 cm cuvette 25 μF 200 Ohms 1400 – 1600 Volts

Suggested Electroporation Systems:

Bio-Rad Micro Pulser #165-2100; Bio-Rad E. coli Pulser #165-2102; Bio-Rad Gene Pulser II #165-2105; BTX ECM630 Electroporation System; Eppendorf Electroporator 2510.

Transformation control reactions are performed with 1 μl (10 pg) of supercoiled pUC19 DNA.

To ensure successful transformation results, the following precautions must be taken:

- Ligation reactions performed with Lucigen's CloneDirect™ Ligation Buffer (included with Lucigen's Cloning or Ligation Kits) must be heat killed at 70°C for 15 minutes before transformation. The ligation reaction can be used immediately after heat inactivation, without purification of the ligation products.
- DNA samples in other buffers must be purified and dissolved in water or a buffer with low ionic strength, such as TE. The presence of salt in the electroporation sample leads to arcing at high voltage, resulting in loss of the cells and DNA.
- Microcentrifuge tubes and electroporation cuvettes must be thoroughly pre-chilled on ice before use. Successful results are obtained with cuvettes from BTX (Model 610), Eppendorf (Cat. #940001005), or BioRad (Cat. #165-2089).
- The cells must be completely thawed **on ice** before use.
- For highest transformation efficiency, use the provided Recovery Medium to resuspend the cells after electroporation. Use of TB, SOC, or other media will result in lower transformation efficiencies.

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Transformation Protocol

1. Have Recovery Medium and 17 mm x 100 mm sterile culture tubes readily available at room temperature (one tube for each transformation reaction). Transformation efficiency may decrease with the use of SOC or other media.
2. Place electroporation cuvettes (0.1 cm gap) and microcentrifuge tubes on ice (one cuvette and one tube for each transformation reaction).
3. Remove cells from the -80°C freezer and place on wet ice until they thaw **completely** (10-20 minutes).
4. When cells are thawed, mix them by tapping gently. Aliquot 25 µl of cells to the chilled microcentrifuge tubes on ice.
5. If using ligation buffer from any Lucigen Cloning Kit, add 1 µl of the heat-denatured ligation reaction to the 25 µl of cells on ice. Failure to heat-inactivate the ligation reaction will prevent transformation. Stir briefly with pipette tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells. Use of more than 2 µl of ligation mix may cause electrical arcing during electroporation.
6. For ligation reactions using other commercial kits, please refer to the manufacturer's instructions.
7. Carefully pipette 25 µl of the cell/DNA mixture into a chilled electroporation cuvette without introducing bubbles. Quickly flick the cuvette downward with your wrist to deposit the cells across the bottom of the well. Electroporate according to the conditions recommended above.
8. Within 10 seconds of the pulse, add 975 µl of Recovery Medium to the cuvette and pipette up and down three times to resuspend the cells. Transfer the cells and Recovery Medium to a culture tube.
9. Place the tube in a shaking incubator at 250 rpm for 1 hour at 37°C.
10. Spread up to 100 µl of transformed cells on plates containing the appropriate antibiotic.
11. Incubate the plates overnight at 37°C.
12. Transformed clones can be further grown in TB or in any other appropriate culture medium.