Lucigen®
Simplifying Genomics

MC1061 F- Electrocompetent Cells

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE

IMPORTANT!
-80 °C Storage Required
Immediately Upon Receipt

Lucigen Corporation 2905 Parmenter St, Middleton, WI 53562 USA
Toll Free: (888) 575-9695 | (608) 831-9011 | FAX: (608) 831-9012
custserv@lucigen.com techserv@lucigen.com www.lucigen.com

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Technical Support

Lucigen is dedicated to the success and satisfaction of our customers. Our products are tested to assure they perform as specified when used according to our recommendations. It is imperative that the reagents supplied by the user are of the highest quality. Please follow the instructions carefully and contact our technical service representatives if additional information is necessary. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

Lucigen Technical Support

Email: techserv@lucigen.com

Phone: (888) 575-9695

Product Guarantee: Lucigen guarantees that this product will perform as specified for one year from the date of shipment. Please avoid using reagents for greater than one year from receipt.

Components & Storage Conditions

MC1061 F- Electrocompetent Cells are packed in Lucigen's DUO format for your convenience:

DUO: 50 µL per vial, enough for two 25-µL reactions

The cells are shipped on dry ice in one container, along with Recovery Medium and supercoiled control pUC19 DNA at 10 pg/ µL. Please refer to Table 1 for a complete listing of MC1061 F- Electrocompetent Cells, efficiencies, and catalog numbers.

Competent Cells require storage at –80 ºC.

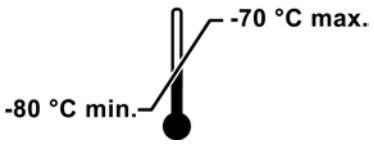

Table 1: **MC1061 F- Competent Cells available from Lucigen**

STRAIN	Format	Efficiency (cfu/µg pUC19)	Transformations		Catalog #	Storage
MC1061 F- Electrocompetent Cells (Pink cap)	DUO	> 4 x 10^{10}	12 24	(6 x 50 µL) (12 x 50 µL)	60514-1 60514-2	**-80 °C**
Recovery Medium	-		12 24 8	(1 x 12 mL) (2 x 12 mL) (8 x 12 mL)	---- ---- ---- 80026-1	**-80 to +20 °C**
*Supercoiled pUC19 DNA (10 pg/µL)	-			(1 x 20 µL)	----	**-80 to -20 °C**

*Provided as a positive control for transformation.

MC1061 F- Competent Cells

MC1061 F- is a non amber-suppressing strain that is commonly used for general cloning and Phage display because of its ability to reach very high transformation efficiencies. It is identical to Lucigen strain SS320, except that it lacks the F' episome that is required for infection by filamentous phage such as M13. The strain can be used for plasmid propagation and phage display, but cannot be used for re-infection.

Transformation Efficiency:
Electrocompetent Cells: $\geq$ 4 x 10^{10} cfu/µg

Genotype

araD139 Δ (araA-leu)7697 Δ(lac)X74 galK16 galE15(GalS) lambda- e14- mcrA0 relA1 rpsL150 (Str^R) *spoT1 mcrB1 hsdR2*

Usage Guidelines

Preparation for Transformation

Transformation is carried out in a 0.1 cm gap cuvette using 25 µL of MC1061 F- Electrocompetent Cells. 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)**	**For 2.0 mm cuvettes**
1.0 mm cuvette 10 µF 600 Ohms 1800 Volts	1.0 mm cuvette 25 µF 200 Ohms 1400 – 1600 Volts	2.0 mm cuvette 25 µF 750 Ohms 2400 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.

Optional transformation control reactions include electroporation 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 ºC for 15 minutes before transformation. The CloneDirect ligation reaction can be used directly 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 can be obtained with cuvettes from many sources including BTX (Model 610), Eppendorf (Cat. #940001005), and 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 SOC or other media will result in lower transformation efficiencies.
- Prepare nutrient agar plus appropriate antibiotic.

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 microfuge 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 pipet 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.

   For ligation reactions using other commercial kits, please refer to the manufacturer's instructions.
6. Carefully pipet 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.
7. Within 10 seconds of the pulse, add 975 µL of Recovery Medium to the cuvette and pipet up and down three times to resuspend the cells. Transfer the cells and Recovery Medium to a culture tube.
8. Place the tube in a shaking incubator at 250 rpm for 1 hour at 37 °C.
9. Spread up to 100 µL of transformed cells on nutrient agar plates containing the appropriate antibiotic.

10. Incubate the plates overnight at 37 °C.

11. Transformed clones can be further grown in any rich culture medium.

Media Recipes

LB Lennox Agar Plates

Per liter: 10 g tryptone
5 g yeast extract
5 g NaCl
15 g agar

Medium for Growth of Transformants
LB Miller

Per liter: 10 g tryptone
5 g yeast extract
10 g NaCl
Add all components to deionized water. Adjust pH to 7.0 with NaOH. Autoclave and cool to 55 °C.

TB

Per liter: 11.8 g tryptone
23.6 g yeast extract
9.4 g dipotassium hydrogen phosphate (anhydrous)
2.2 g potassium dihydrogen phosphate (anhydrous)
0.4% glycerol
Add all components to deionized water.. Autoclave and cool to 55 °C.

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