Lucigen®
Simplifying Genomics

Phage Display Electrocompetent Cells

TG1
SS320 (MC1061F´)
ER2738

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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

All strains of Phage Display Electrocompetent Cells are pre-dispensed as DUOs (50 µL aliquots), sufficient for two transformation reactions of 25 µL each.

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

Electrocompetent Cells require storage at –80 ºC.

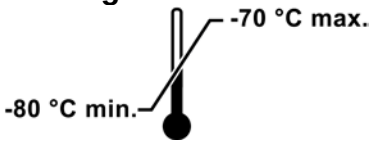

Table 1: **Phage Display Electrocompetent Cells and Components**

STRAIN	Cap Color	Efficiency (cfu/µg pUC19 DNA)	Transformations	Catalog #	Storage
TG1 DUOs	Yellow	$\geq 4 \times 10^{10}$	12 (6 x 50 µL) 24 (12 x 50 µL)	60502-1 60502-2	**-80 °C**
SS320 DUOs (MC1061 F´)	Red	$\geq 4 \times 10^{10}$	12 (6 x 50 µL) 24 (12 x 50 µL)	60512-1 60512-2	**-80 °C**
ER2738 DUOs	Blue	$\geq 2 \times 10^{10}$	12 (6 x 50 µL) 24 (12 x 50 µL)	60522-1 60522-2	**-80 °C**
Phage Display Electrocombo Pack	Yellow Red Blue	$\geq 2 - 4 \times 10^{10}$	12 (6 x 50 µL) 4 transformations of each of 3 strains	60500-0	**-80 °C**
Recovery Medium	White		(1 x 12 mL) (2 x 12 mL) (8 x 12 mL)	---- ---- 80026-1	**-80 to +25 °C**
*Supercoiled pUC19 DNA (10 pg/µL)	Clear		(1 x 20 µL)	----	**-80 to -20°C**

*Provided as a control for transformation—use 1 µL (10 pg) for transformation.

Description & Uses

TG1 Electrocompetent Cells deliver $\geq 4 \times 10^{10}$ cfu/µg of DNA and are particularly useful for phage display protein expression. TG1 cells are also suitable for M13 phage work, general cloning, blue/white screening and protein expression.

SS320 (MC1061 F´) Electrocompetent Cells deliver $\geq 4 \times 10^{10}$ cfu/µg of DNA and are particularly useful for phage display protein expression. SS320, also known as MC1061 F´ cells, are also suitable for M13 phage work, general cloning, blue/white screening and protein expression.

Phage Display Electrocompetent Cells

ER2738 Electrocompetent Cells deliver $\geq$ 2 x 10^{10} cfu/µg of DNA and are particularly useful for phage display protein expression. ER2738 cells are also suitable for M13 phage work, general cloning, blue/white screening and protein expression.

Genotype

TG1: [F´ *traD36 proAB lacI^{q}Z* ∆*M15*] *supE thi-1* ∆(*lac-proAB*) ∆(*mcrB-hsdSM*)5(r_K^- m_K^-)

SS320 (MC1061F´): [F'*proAB*$^+$*lacI^{q}lacZ*∆M15 *Tn10* (tet^r)] *hsdR mcrB araD139* ∆(*araABC-leu*)7679 ∆*lac*X74 *galUgalK rpsL thi*

ER2738: [F´*proA*$^+$*B*$^+$ *lacI^q* ∆(*lacZ*)M15 *zzf::Tn10* (tet^r)] *fhuA2 glnV*∆(*lac-proAB*) *thi-1*∆(*hsdS-mcrB*)5

Preparation for Transformation

Transformation is carried out in a 0.1 cm gap cuvette using 25 µL of 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)
1.0 mm cuvette 10 µF 600 Ohms 1800 Volts		1.0 mm 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.

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 must be heat killed at 70 ºC for 15 minutes before transformation. The ligation reaction can be used directly after heat inactivation, without purification of the ligation products.
- DNA samples 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). Users have reported much lower transformation efficiencies using cells with Invitrogen cuvettes (Cat. # 65-0030).
- 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 or other media will result in lower transformation efficiencies.

Cells may be plated on LB or other common media.

Transformation Protocol for Cells

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 Electrocompetent Cells from the -80 °C freezer and place on wet ice until they thaw **completely** (10-15 minutes).

4. When the cells are thawed, mix them by tapping gently. Aliquot 25 µL of cells into the chilled microcentrifuge tubes on ice.

5. If using ligation buffer from any Lucigen cloning or ligation 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 LB (or other nutrient media) agar plates containing the appropriate antibiotic.

10. Incubate the plates overnight at 37 °C.

11. Transformed clones can be further grown in TB or in any other 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.
LB Lennox Agar is used to maximize colony size. Cells may be plated on LB or other common media—colonies will be noticeably smaller upon comparison but adequate for the vast majority of intents and purposes.