

**Please note:** The concentration of the included pUC19 has been changed from 1ng/μl to 10pg/μl. Do not dilute the plasmid before performing the transformation positive control. Please contact Lucigen if you have any questions.

# **Phage Display Electrocompetent Cells: TG1, SS320 (MC1061F'), and ER2738**

**IMPORTANT!  
-80°C Storage Required  
Immediately Upon Receipt**

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# Phage Display Electrocompetent Cells

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# Phage Display Electrocompetent Cells

## 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, YT Agar and supercoiled control pUC19 DNA at 10 pg/µl. Please refer to Table 1 for a complete listing of Phage Display Electrocompetent Cells, efficiencies, and catalog numbers.

**Electrocompetent Cells require storage at –80° C.**

**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	60500-1	-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
YT Agar (powder)	----		1 Packet 5 Packet 10 Packet	---- 60025-1 60025-2	ambient

\*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.

**ER2738 Electrocompetent Cells** deliver  $\geq 2 \times 10^{10}$  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.

# Phage Display Electrocompetent Cells

## Genotype

TG1: [F' *traD36 proAB lacI<sup>f</sup>Z ΔM15*] *supE thi-1 Δ(lac-proAB) Δ(mcrB-hsdSM)5(r<sub>K</sub><sup>-</sup> m<sub>K</sub><sup>-</sup>)*

SS320 (MC1061F'): [F'*proAB<sup>+</sup>lacI<sup>f</sup>lacZΔM15 Tn10 (tet<sup>r</sup>)*] *hsdR mcrB araD139 Δ(araABC-leu)7679 ΔlacX74 galUgalK rpsL thi*

ER2738: [F'*proA<sup>+</sup>B<sup>+</sup> lacI<sup>f</sup> Δ(lacZ)M15 zzf::Tn10 (tet<sup>r</sup>)*] *fhuA2 glnVΔ(lac-proAB) thi-1Δ(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	1.0 mm cuvette
10 μF	25 μF
600 Ohms	200 Ohms
1800 Volts	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°C 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.
- Prepare YT Agar from powder included in kit plus appropriate antibiotic (see Media Recipes on page 6). YT Agar is provided 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.

# Phage Display Electrocompetent Cells

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## 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 YT (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.

# Phage Display Electrocompetent Cells

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## Media Recipes

### TB Culture Medium for Growth of Transformants

Per liter:

- 11.8 g Bacto-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 except glycerol to deionized water. Autoclave and cool to 55°C. Immediately before use, add 8 ml of filter-sterilized 50% glycerol.

### YT Agar Plates\*

Add the YT Agar powder provided with the kit to 500 ml of deionized water. Autoclave and cool to 55°C. Add the appropriate filter-sterilized antibiotic to the cooled medium (e.g., 15 mg kanamycin for kanamycin-resistant transformants; 50 mg ampicillin or carbenicillin for ampicillin-resistant transformants).

Temperatures of >55°C may destroy the antibiotics. Do NOT add antibiotics to hot media!

YT agar composition (per liter): 5 g yeast extract, 8 g tryptone, 5 g NaCl, 15 g agar, pH 7.0. Pour approximately 20-25 ml per petri plate.

For blue/white screening, add 3 ml 100mM IPTG and 10 ml 2% X-gal per liter to the molten agar at 55°C before pouring.

Additional YT Agar is available from Lucigen as catalog number 60025-1 (5 packets). Each packet makes 500 ml.

\*YT Agar is provided 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.

## Related Lucigen Products

- BigEasy® Linear Cloning Kit
- CloneSmart® Blunt Cloning Kits
- DNATerminator® End Repair Kit
- PCRTerminator® End Repair Kit
- UltraClone™ DNA Ligation & Transformation Kits
- CloneDirect™ Rapid Ligation Kit
- ClonePlex® Library Construction Kit
- pEZSeq™ Blunt Cloning Kits
- *E. coli*® EXPRESS Electrocompetent Cells
- OverExpress™ Competent Cells