Manual

Phage Display Electrocompetent Cells

For Research Use Only. Not for use in diagnostic procedures.

IMPORTANT:
-80 ºC storage required
immediately upon receipt

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

Manual

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Contents

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1. Product description

ER2738 Electrocompetent Cells deliver ≥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.

SS320 (MC1061 F´) Electrocompetent Cells deliver ≥4 x 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.

TG1 Electrocompetent Cells deliver ≥4 x 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.

2. Product specifications

Strain	Transformation efficiency	Genotype
ER2738	≥2 x 10^{10} cfu/µg of pUC DNA	[F′*proA*$^{+}$*B*$^{+}$ *lacI*^{q} Δ*(lacZ)*M15 *zzf::Tn10* (tet^{r})*] fhuA2 glnV*Δ*(lac-proAB) thi-1*Δ*(hsdS-mcrB)5*
SS320 (MC1061F´)	≥4 x 10^{10} cfu/µg of pUC DNA	[F′*proA*$^{+}$*B*$^{+}$ *lacI*^{q}*lacZ*ΔM15 *Tn10* (tet^{r})*] hsdR mcrB araD139* Δ*(araABC-leu)7679* Δ*lacX74 galUgalK rpsL thi*
TG1	≥4 x 10^{10} cfu/µg of pUC DNA	[F´ *traD36 proA*$^{+}$*B*$^{+}$ *lacI*^{q}*Z* Δ*M15*] *supE thi-1* Δ*(lac-proAB)* Δ*(mcrB-hsdSM)5(rK*$^{-}$ *mK*$^{-}$*)*

3. Product designations and kit components

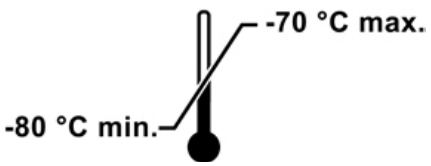
Product	Kit size	Catalogue number	Reagent description	Part number	Volume
ER2738 Electrocompetent Cells (blue cap)	12 reactions (DUOS*)	60522-1	ER2738 Electrocompetent Cells	F96633	12 (6 x 50 µL)
			Transformation Control pUC 19**	F92078-1	(1 x 20 µL)
			Recovery Media***	F98226-1	(1 x 12 mL)
	24 reactions (DUOS*)	60522-2	ER2738 Electrocompetent Cells	F96633	24 (12 x 50 µL)
			Transformation Control pUC 19**	F92078-1	(1 x 20 µL)
			Recovery Media***	F98226-1	(2 x 12 mL)
SS320 (MC1061 F´) Electrocompetent Cells (red cap)	12 reactions (DUOS*)	60512-1	SS320 (MC1061 F′) Electrocompetent Cells	F96632	12 (6 x 50 µL)
			Transformation Control pUC 19**	F92078-1	(1 x 20 µL)
			Recovery Media***	F98226-1	(1 x 12 mL)
	24 reactions (DUOS*)	60512-2	SS320 (MC1061 F′) Electrocompetent Cells	F96632	24 (12 x 50 µL)
			Transformation Control pUC 19**	F92078-1	(1 x 20 µL)
			Recovery Media***	F98226-1	(2 x 12 mL)

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
TG1 Electrocompetent Cells (yellow cap)	12 reactions (DUOS*)	60502-1	TG1 Electrocompetent Cells	F96595	12 (6 x 50 µL)
			Transformation Control pUC 19**	F92078-1	(1 x 20 µL)
			Recovery Media***	F98226-1	(1 x 12 mL)
	24 reactions (DUOS*)	60502-2	TG1 Electrocompetent Cells	F96595	24 (12 x 50 µL)
			Transformation Control pUC 19**	F92078-1	(1 x 20 µL)
			Recovery Media***	F98226-1	(2 x 12 mL)

*Phage Display Electrocompetent Cells are packaged as DUOS in 50 µL aliquots, sufficient for two transformations per tube.
**Supercoiled pUC19 DNA (10 pg/µL; clear cap) is provided as a control for transformation and should be stored at -20 to -80 °C. Use 1 µL (10 pg) for transformation.
***Recovery Media (white cap) should be stored at -20 to -80 °C. It is also available separately as Catalogue #80026-1 (96 mL; 8 x 12 mL)..

4. Storage conditions

Electrocompetent cells require storage at -80 °C



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 Table 1 below. Typical time constants are 3.5 to 4.5 msec.

Condition	Optimal setting	Alternate settings (~ 20-50% lower efficiencies)
Cuvette gap	1.0 mm	1.0 mm
Voltage	1,800 V	1,400-1,600 V
Capacitance	10 µF	25 µF
Impedance	600 Ohms	200 Ohms
Electroporator	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.	

Table 1. Electroporation conditions for Phage Display Electrocompetent Cells

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.

Cells may be plated on LB or other common media.

6. 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 LGC Biosearch Technologies 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 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.
   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.

6.1 Media recipes

LB Lennox agar plates

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

Medium for growth of transformants

LB Miller

Per litre:	10 g	tryptone
	5 g	yeast extract
	5 g	NaCl

Add all components to deionised water. Adjust pH to 7.0 with NaOH. Autoclave and cool to 55 °C.

TB

Per litre:	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 deionised water. Autoclave and cool to 55 °C.

LB Lennox agar is used to maximise 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.

7. Technical support and product guarantee

Biosearch Technologies 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.

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

Product guarantee: Biosearch Technologies 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.

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