Manual

TransforMax EC100 Chemically Competent *E. coli*

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

TransforMax™ EC100™ Chemically Competent *E. coli* is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

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TransforMax EC100 Chemically Competent *E. coli*

1. Introduction

TransforMax™ EC100™ Chemically Competent *E. coli* are useful for primary cloning as well as subcloning applications.

The cells are provided in 50 µL aliquots (1 transformation per tube) for ease of use. The transformation efficiency of the TransforMax EC100 Chemically Competent cells is >1 x 10^8 cfu/µg DNA using pUC19.

Relevant phenotype

- Compatible with vectors expressing the *LacZ'* α-complementing peptide for 'blue/white' screening of recombinants.
- Restriction minus for efficient cloning of methylated DNA.
- Accepts large clones.
- Endonuclease minus (*end*A1) to ensure high yields of plasmid clones.
- Recombination minus (*rec*A1) to ensure the stability of large cloned inserts.

2. Product specifications

Transformation efficiency:	Electrocompetent cells: > 1 x 10^8 cfu/µg of supercoiled DNA*
Genotype	F$^-$ *mcr*A Δ*(mrr-hsd*RMS-*mcr*BC*)* ϕ80d*lacZ*ΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139 Δ*(ara, leu)*7697 *gal*U *gal*K λ$^-$ *rps*L *nup*G
Quality control:	TransforMax EC100 Chemically Competent *E. coli* yield >1 x 10^8 transformants per microgram of supercoiled DNA. Transformation efficiency is determined using 10 pg of pUC19 and the Standard transformation procedure described. TransforMax EC100 Chemically Competent *E. coli* are tested to be free of contaminating DNA rendering resistance to ampicillin, tetracycline, kanamycin and chloramphenicol.

3. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
TransforMax EC100 Chemically Competent *E. coli*	10 x 50 µL	CC02810	TransforMax EC100 Chemically Competent *E. coli*	SS001003-D	10 x 50 µL
			pUC19 DNA *	SS000200-D	10 µL

* Each is supplied with 10 µL (100 pg/µL) of pUC19 Control DNA in TE Buffer.

4. Storage conditions

Store TransforMax EC100 *E. coli* cells at -70 °C and the pUC19 Control DNA at either -20 °C or -70 °C. Do not thaw and refreeze the cells. Refreezing will result in significantly reduced transformation efficiency.

5. Transformation procedures

Two procedures for transforming the TransforMax EC100 Chemically Competent *E. coli*. are presented. The Standard transformation procedure will provide the highest transformation efficiency. The 5 minute transformation procedure is more rapid but may yield 10-fold or more lower transformation

efficiency. The 5 minute transformation procedure should only be used with ampicillin selection of the clones. Both procedures were written for transformation of 50 µL of TransforMax EC100 Chemically Competent *E. coli*.

NOTE: *Once thawed, do not refreeze the cells.*

A different volume of cells can also be used based on the experiences and needs of the user.

Standard transformation procedure

1. Prepare 250 µL of SOC medium[1] for each transformation to be performed. Maintain the media at room temperature.
2. Heat a water bath or other temperature-controlled apparatus to 42 °C.
3. Thaw the appropriate number of tubes of TransforMax EC100 Chemically Competent *E. coli* cells on ice. Mix by gentle tapping. Use the cells immediately.
4. Transfer 1-5 µL of DNA or ligation reaction into each tube. Cap the tubes and incubate on ice for 5-30 minutes.
5. Transfer the tubes to 42 °C and heat shock for 30 seconds.
6. Transfer the cells back to ice and cool for 2 minutes.
7. Remove the cover of the tubes and add 250 µL of SOC Media.
8. Recover the cells by incubating at 37 °C for 60 minutes with horizontal shaking (e.g. 225 rpm).
9. Plate the cells on the appropriate media and antibiotic, and grow overnight at 37 °C.
10. Dilute and plate the cells on appropriate medium and antibiotic. For cells transformed with the control pUC19 DNA, plate on LB agar containing 100 µg/mL of ampicillin. The remaining cell outgrowth can be stored at 4 °C in the event additional cell dilutions are plated.

5 minute transformation procedure

The rapid 5 minute transformation procedure may yield 10-fold or more lower transformation efficiency than the Standard transformation procedure described above. Importantly, only selection with ampicillin can be used with the 5 minute transformation procedure.

1. Thaw the appropriate number of tubes of TransforMax EC100 Chemically Competent *E. coli* cells on ice. Mix by gentle tapping. Use the cells immediately.
2. Transfer 3-5 µL of DNA or ligation reaction into each tube. Cap the tubes and incubate on ice for 5 minutes.
3. Spread the entire cell/DNA mixture onto a pre-warmed LB+ampicillin (100 µg/mL) plate and grow overnight at 37 °C.

6. Reference

1. Hanahan D, 1983, *J. Mol. Biol.*, 166, 557.

7. Further support

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

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