Manual

Phage T1-Resistant TransforMax EPI300-T1^{R} Electrocompetent *E. coli*

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

Phage T1-Resistant TransforMax™ EPI300™-T1^{R} Electrocompetent *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.

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

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1. Product description

Phage T1-Resistant TransforMax EPI300-T1^R Electrocompetent *E. coli* have been specifically engineered for use with LGC, Biosearch Technologies' CopyControl™ Cloning Systems.* The cells contain the *trf*A gene, whose protein product is required for initiation of replication from the *ori*V origin of replication contained on the CopyControl vectors. The *trf*A gene is under tightly regulated control of an inducible promoter. When grown on standard LB plates or in LB or SOC culture medium, expression of the *trf*A gene is repressed. Addition of the CopyControl Induction Solution (provided) induces expression of the *trf*A gene and subsequent utilisation of the *ori*V origin of replication and high copy amplification of the CopyControl clones. In addition, these cells are resistant to bacteriophage T1 and T5 infections (*ton*A genotype).

Benefits

- *trf*A gene under tightly regulated control of an inducible promoter for copy number control of CopyControl clones.
- *ton*A for resistance to bacteriophage T1 and T5 infections.
- Supports blue/white screening of vectors.
- Readily accepts large DNAs for construction of large-insert libraries.
- Restriction minus [*mcr*A Δ(*mrr-hsd*RMS-*mcr*BC)] for efficient cloning of methylated (e.g. mammalian genomic) DNA.
- 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:	Greater than 10*10* cfu/µg DNA using 10 pg of pUC19 and an Eppendorf Multiporator with setting of 2.5 KV at 5 milliseconds, fast charge rate using 2 mm cuvettes.
Genotype	F$^-$ *mcrA* Δ*(mrr-hsd*RMS-*mcr*BC*)* ϕ80d*lac*ZΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139 Δ*(ara, leu)*7697 *gal*U *gal*K λ$^-$ *rps*L *nup*G *trf*A *ton*A *dhfr*
Quality control:	Phage T1-Resistant TransforMax EPI300-T1^R *E. coli* cell types are tested: • for induced expression of the *trf*A gene using transformants harboring a plasmid with an *ori*V origin of replication. • to be free of contaminating DNA rendering resistance to ampicillin, tetracycline, kanamycin and chloramphenicol. • for bacteriophage T1 resistance: genotypically, by diagnostic PCR of the *ton*A gene, phenotypically by resistance to bacteriophage T5 infection.

3. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
TransforMax EPI300-T1^{R} Electrocompetent *E. coli*	10 x 100 µL**	EC02T110	TransforMax EPI300-T1R Electrocompetent *E. coli*	SS001011	10 x 100 µL
			pUC19 DNA†	SS000200-D	10 µL
			CopyControl Induction Solution‡	SS000023-DCON	

** 10 electroporations.

† Supplied with 10 µL (100 pg/µL) of pUC19 Control DNA in TE Buffer.

‡ CopyControl Induction Solution is also available separately as Catalogue #CCIS125 (25 mL)

4. Storage conditions

Store TransforMax EPI300 *E. coli* cells at -70 °C. The CopyControl Induction Solution and the pUC19 Control DNA can be stored at either -20 °C or -70 °C. Warm the CopyControl Induction Solution to room temperature and mix thoroughly before use.

5. Electroporation of Phage T1-Resistant TransforMax EPI300-T1^{R} Electrocompetent *E coli*

1. DNA should be in water or very low salt buffer (e.g. TE Buffer: 10 mM Tris-HCl [pH 7.5], 1 mM EDTA) to prevent arcing during electroporation. The pUC19 Control DNA is provided in TE at 100 pg/µL. If running a transformation control, dilute the pUC19 Control DNA 1:10 (to a final concentration of 10 pg/µL) with sterile, deionised water and use 1 µL for electroporation.
2. Prepare 1 mL of SOC medium (do not include antibiotic in the medium) for each electroporation to be performed. This medium will be used for post-electroporation outgrowth of transformed cells. Maintain the medium at room temperature.
3. Pre-chill electroporation cuvettes and 1.5 mL tubes on ice.
4. Set-up the electroporation device according to the manufacturer's recommendations for bacterial (*E. coli*) electroporation.
5. Thaw TransforMax EPI300-T1^{R} Electrocompetent *E. coli* cells on ice. Mix by gentle tapping or vortexing. Use the cells immediately. Unused cells can be refrozen at -70 °C.
   NOTE: *Refrozen cells may have reduced transformation efficiency.*
6. Transfer the desired amount of DNA and 50 µL of cells to a pre-chilled microcentrifuge tube.
   NOTE: *A smaller volume of cells can be used based on the needs and experiences of the user. Mix the cells and DNA by pipetting up and down 2-3 times.*
7. Transfer the cell/DNA mix to the electroporation cuvette. Be sure that there are no air bubbles in the cuvette. Wipe the cuvette of any condensation. Place into the electroporator and apply the electric pulse at the manufacturer's recommendations for bacterial (*E. coli*) electroporation.
8. Immediately after electroporation, add 950 µL of room temperature SOC medium[1] to the cuvette. Mix gently by pipetting up and down 2-3 times.
9. Transfer the cells to a 15 mL tube and incubate at 37 °C with shaking at 220-230 rpm for one hour to recover the cells and allow expression of the antibiotic resistance marker.

10. Dilute and plate the cells on appropriate medium (e.g. LB agar plates) 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.

Control (Optional): Dilute the control reaction 1:20 and plate 100 µL (equivalent to 0.05 pg DNA) to LB-ampicillin (100 µg/mL) plates. If 250 colonies are observed on the plate, the transformation efficiency is 5 x 10^9 cfu/µg or [(250 cfu/0.05 pg DNA) x (10^6 pg/µg)].

6. Induction of CopyControl fosmid to high copy number

CopyControl fosmid clones grown in TransforMax EPI300-T1^R cells can be amplified to 10-50 copies per cell. The induction process can be done in any culture volume desired depending on the needs of the user. Generally, a 1 mL induced culture will provide a sufficient amount of DNA for most applications including sequencing and fingerprinting. Here we provide the procedure for amplifying the clones in 1 mL, 5 mL and 50 mL cultures.

Growth media for CopyControl fosmid clones

LB + chloramphenicol (12.5 µg/mL)

1. Add 5 mL of the Growth media to 15 mL tubes for each fosmid clone that will be induced to high copy number.
2. Individually inoculate the media with a small portion of the desired fosmid or PCR clones grown on an overnight plate.
3. Grow the cultures overnight at 37 °C with shaking. These cultures will be used as inocula for the copy number amplification procedure.
4. From the table below, combine the appropriate volumes of fresh Growth media, the overnight culture and the CopyControl Induction Solution for the desired volume of induction culture. Aeration of the induction cultures is critical. Therefore, to maximise the surface area of the culture solution in the tube, perform the induction in the largest volume tubes that reasonably meets your needs and resources. For example, induce clones to high copy number in 1 mL of culture, using 1.5 mL tubes or larger, 5 mL cultures in 15 mL tubes and 50 mL cultures in 125 mL flasks.

Total volume of clone induction culture	Volume of fresh LB + chloramphenicol (12.5 µg/mL)	Volume of overnight 5 mL culture	Volume of 1000X CopyControl Induction Solution[§]
1 mL	800 µL	200 µL	1 µL
5 mL	4.5 mL	500 µL	5 µL
50 mL	45 mL	5 mL	50 µL

[§] Mix thoroughly after thawing.

5. Vigorously shake the tubes at 37 °C for 5 hours. Aeration is critical! Shake the tubes in a manner that will maximise aeration of the cultures (for example 1.5 mL tubes can be taped horizontally to the shaking table).
6. Centrifuge the cells and purify the DNA by your standard lab methods.

7. References

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

8. 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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