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# Phage T1-Resistant TransforMax™ EC100™-T1$^{R}$ Electrocompetent *E. coli*

Cat. No. EC0205T1

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## 1. Introduction

Phage T1-Resistant TransforMax™ EC100™-T1$^R$ Electrocompetent *E. coli* are very versatile cells that are useful for most cloning applications. The cells feature very high transformation efficiencies and are resistant to bacteriophage T1 and T5 infections (*ton*A genotype).

The Phage T1-Resistant TransforMax EC100-T1$^R$ Electrocompetent *E. coli* should not be used with Epicentre's CopyControl™ Cloning Systems. Use the Phage T1-Resistant TransforMax EPI-300™-T1$^R$ Electrocompetent *E. coli* with the CopyControl Cloning Systems.

**Benefits:**

- *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. Kit contents

| Cat. # | Quantity |
|---|---|
| **Phage T1-Resistant TransforMax™ EC-100™-T1$^R$ Electrocompetent *E. coli*:** | |
| EC0205T1 | 5 x 100 µl |
| Supplied with 10 µl (100 pg/µl) of pUC19 Control DNA in TE Buffer. | |

## 3. Product Specifications

**Storage:** Store Phage T1-Resistant TransforMax EC100-T1$^R$ *E. coli* cells at –70°C and the pUC-19 Control DNA at either –20°C or –70°C.

**Genotype:**

F$^-$ *mcr*A 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 *ton*A.

**Quality Control:**

- Phage T1-Resistant TransforMax EC100-T1$^R$ Electrocompetent *E. coli* have a transformation efficiency of >1 x $10^{10}$ cfu/µg DNA using 10 pg of pUC19 and an Eppendorf Multiporator with setting of 2.5 KV at 5 msecs., fast charge rate using 2 mm cuvettes.
- Phage T1-Resistant TransforMax EC100-T1$^R$ Electrocompetent *E. coli* are tested to be free of contaminating DNA rendering resistance to ampicillin, tetracycline, kanamycin, and chloramphenicol.

- Phage T1-Resistant TransforMax EC100-T1$^R$ Electrocompetent *E. coli* are tested for bacteriophage T1 resistance: genotypically, by diagnostic PCR of the *ton*A gene, and phenotypically by resistance to bacteriophage T5 infection.

### Electroporation of Phage T1-Resistant TransforMax EC100-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, deionized 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 EC100-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 [Hanahan, D., (1983) *J. Mol. Biol.*, **166**, 557] 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 1 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 pUC19 Control 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)].



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