One Shot® OmniMAX™ 2 T1 Phage-Resistant Cells

Cat. No. C8540-03 Size: 20 reactions

Shipping and Storage
The One Shot® OmniMAX™ 2-T1^R Chemically Competent *E. coli* kit is shipped on dry ice. Upon receipt, store at -80°C.

Caution
This product contains Dimethyl Sulfoxide (DMSO), a hazardous material. Review the Material Safety Data Sheet before handling.

Kit Contents
Each kit contains the reagents listed below. **Transformation efficiency is greater than 5 x 10^9 cfu/µg DNA.**

Item	Composition	Amount
S.O.C. Medium (store at room temperature or +4°C)	2% Tryptone, 0.5% Yeast Extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose	6 ml
OmniMAX™ 2-T1^R Cells	---	21 x 50 µl
pUC19 Control DNA	10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8	50 µl

Genotype
F′ {*pro*AB$^+$ *lac*I^q *lac*ZΔM15 *Tn*10(Tet^R) Δ(*ccd*AB)} *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80(*lac*Z)ΔM15 Δ(*lac*ZYA-*arg*F) U169 *end*A1 *rec*A1 *sup*E44 *thi*-1 *gyr*A96 *rel*A1 *ton*A *pan*D

Information for European Customers
The OmniMAX™ 2-T1^R strain is genetically modified and carries the F′ episome containing *pro*AB$^+$ *lac*I^q *lac*ZΔM15 *Tn*10 Δ(*ccd*AB). As a condition of sale, use of this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

Product Qualification
Competent cells are tested for transformation efficiency using the control plasmid included in the kit. Transformed cultures are plated on LB plates containing 100 µg/ml ampicillin and the transformation efficiency is calculated. Test transformations are performed in duplicate. Transformation efficiency should be greater than 5 x 10^9 cfu/µg plasmid DNA. In addition, untransformed cells are tested for appropriate antibiotic sensitivity, the absence of phage contamination, and resistance to phage T5 (a standard test for resistance to phage T1).

Features of the Strain
The OmniMAX™ 2-T1^R *E. coli* strain possesses several features that make it an ideal strain to use for most cloning applications. These features include:

- Δ(*ccd*AB) for sensitivity to the toxic effects of the *ccd*B gene product, allowing negative selection of vectors containing the *ccd*B gene
- High transformation efficiency (>5 x 10^9 cfu/µg DNA)
- Elimination of *mcr*A, *mrr*, *mcr*BC, and *hsd*RMS restriction systems to allow construction of more representative genomic libraries (Blumenthal, 1989; Grant *et al.*, 1990)
- *ton*A genotype to confer resistance to T1 and T5 phage

General Guidelines
Perform the following before starting the transformation procedure:

1. Handle competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Thaw One Shot® competent cells on ice, and transform cells immediately following thawing. After adding DNA, mix by swirling or tapping the tube gently. **Do not mix cells by pipetting**.
2. One Shot® OmniMAX™ 2-T1^R cells are *lac*I^q and require IPTG to induce expression from the *lac* promoter. Spread 40 µl of 100 mM IPTG on top of the agar. Let the IPTG diffuse into the agar for approximately 1 hour. If blue/white screening is required to select for transformants, spread 40 µl of 40 mg/ml X-Gal in dimethylformamide in addition to IPTG on top of the agar. Let the X-Gal and IPTG diffuse into the agar for approximately 1 hour.

Transforming Competent Cells

Perform the following before starting the transformation procedure:

- Equilibrate a water bath to 42°C.
- Warm the vial of S.O.C. Medium (supplied with the kit) and LB Medium to room temperature.
- Spread IPTG or IPTG and X-Gal onto LB agar plates containing antibiotic, if desired.
- Warm the selective plates in a 37°C incubator for 30 minutes (use one plate for each transformation). If you are including the pUC19 control, make sure that you have one LB agar plate containing 100 µg/ml ampicillin.

Transformation Procedure

Use the following procedure to transform One Shot® OmniMAX™ 2-T1^R chemically competent *E. coli*. We recommend including the pUC19 control plasmid DNA supplied with the kit in your transformation experiment to verify the efficiency of the competent cells. **Do not** use these cells for electroporation.

1. Thaw, on ice, one vial of One Shot® OmniMAX™ 2-T1^R chemically competent cells for each transformation.
2. Add 1 to 5 µl of the DNA (10 pg to 100 ng) into a vial of One Shot® cells and mix gently. **Do not mix by pipetting up and down.** If you are transforming the pUC19 control, add 1 µl (10 pg) into a separate vial of One Shot® cells and mix gently.
3. Incubate the vial(s) on ice for 30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking.
5. Remove the vial(s) from the 42°C bath and place them on ice for 2 minutes.
6. Add 250 µl of pre-warmed S.O.C. Medium to each vial.
7. Cap the vial(s) tightly and shake horizontally at 37°C for 1 hour at 225 rpm in a shaking incubator.
8. Before plating, dilute the transformation mix 1:50 into LB Medium (*e.g.* remove 10 µl of the transformation mix and add to 490 µl of LB Medium).
9. Spread 25-100 µl of the diluted transformation mix on a pre-warmed selective plate. Store the remaining undiluted and diluted transformation mix at +4°C. Additional cells may be plated out the next day, if desired.
10. Invert the plate(s) and incubate at 37°C overnight.
11. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

Calculating Transformation Efficiency

Use the following formula to calculate the transformation efficiency as transformants (in cfu) per µg of plasmid DNA, where DF is the dilution factor.

$$\frac{\text{\# of colonies}}{\text{10 pg transformed DNA}} \times \frac{10^6 \text{ pg}}{\mu\text{g}} \times \frac{\text{300 µl total transformation volume}}{\text{X µl plated}} \times \text{50 (DF)} = \frac{\text{\# transformants}}{\mu\text{g plasmid DNA}}$$

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References

Blumenthal, R. M. (1989). Cloning and Restriction of Methylated DNA in *Escherichia coli*. Focus *11*, 41-46.

Grant, S. G., Jessee, J., Bloom, F. R., and Hanahan, D. (1990). Differential Plasmid Rescue from Transgenic Mouse DNAs into Escherichia coli Methylation-restriction Mutants. Proc. Natl. Acad. Sci. USA *87*, 4645-4649.