invitrogen™

One Shot® Mach1™-T1R Chemically Competent *E. coli*

Cat. No. C8620-03 **Size: 20 reactions**

Shipping and Storage

The One Shot® Mach1™-T1R Chemically Competent *E. coli* kit is shipped on dry ice. Upon receipt, store at -80°C.

Kit Contents

Each kit contains the reagents listed below. **Transformation efficiency is greater than 1 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
Mach1™-T1R 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- ϕ80(*lac*Z)ΔM15 Δ*lac*X74 *hsd*R(r_K-m_K+) Δ*rec*A1398 *end*A1 *ton*A

Information for Non-U.S. Customers

For European Customers

The Mach1™-T1R *E. coli* strain is genetically modified to carry the *lac*ZΔM15 *hsd*R *lac*X74 *rec*A *end*A *ton*A genotype. As a condition of sale, 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.

For All Non-U.S. Customers

The parental strain of Mach1™-T1R *E. coli* is the non-K-12, wild-type W strain (ATCC #9637, S. A. Waksman). Although the parental strain is generally classified as Biosafety Level 1 (BL-1), we recommend that you consult the safety department of your institution to verify the Biosafety Level.

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 1 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 that demonstrates resistance to phage T1).

Features of the Strain

The Mach1™-T1R *E. coli* strain is modified from the wild-type W strain (ATCC #9637, S. A. Waksman) and has a faster doubling time compared to other standard cloning strains. With Mach1™-T1R cells, you can visualize colonies 8 hours after plating on ampicillin selective plates. You can also prepare plasmid DNA 4 hours after inoculating a single, overnight-grown colony in the selective media of choice. Note that this feature is not limited to ampicillin selection. Additional key features of the Mach1™-T1R *E. coli* strain include:

- *lac*ZΔM15 for blue/white color screening of recombinants
- *hsd*R mutation for efficient transformation of unmethylated DNA from PCR applications
- Δ*rec*A1398 mutation for reduced occurrence of homologous recombination in cloned DNA
- *end*A1 mutation for increased plasmid yield and quality
- *ton*A mutation to confer resistance to T1 and T5 phage

General Guidelines

Follow these guidelines when using One Shot® Mach1™-T1R Chemically Competent *E. coli.*

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® Mach1™-T1R cells do not require IPTG to induce expression from the *lac* promoter. If blue/white screening is required to select for transformants, spread 40 µl of 40 mg/ml X-Gal in dimethylformamide on top of the agar. Let the X-Gal 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) to room temperature.
- Spread 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. **Note:** For optimal growth of Mach1™-T1^R *E. coli* cells, it is essential that selective plates are prewarmed to 37°C prior to spreading.

Transformation Procedure

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® Mach1™-T1^R Chemically Competent *E. coli* 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 room temperature 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. Spread 25-100 µl of the transformation mix on a **prewarmed** selective plate. Store the remaining transformation mix at +4°C. Additional cells may be plated out the next day, if desired.
9. Invert the plate(s) and incubate at 37°C. If you are using ampicillin selection, visible colonies should appear within 8 hours, and blue/white screening can be performed after 12 hours. If you are selecting transformants with an antibiotic other than ampicillin, incubate plates overnight.
10. Select overnight-grown colonies and analyze by plasmid isolation, PCR, or sequencing. For plasmid isolation, inoculate a single, overnight-grown colony in 2 ml of **prewarmed** selective media (*e.g.* LB + ampicillin, LB + kanamycin, LB + Zeocin™, *etc.*). For optimal results, we recommend inoculating as much of the single colony as possible. Shake at 37°C for 4 hours before isolating the plasmid.

Calculating Transformation Efficiency

Use the following formula to calculate the transformation efficiency as transformants (in cfu) per µg of plasmid DNA.

$$\frac{\text{\# of colonies}}{\text{10 pg transformed DNA}} \times \frac{10^6 \text{ pg}}{\text{µg}} \times \frac{\text{300 µl total transformation volume}}{\text{X µl plated}} = \frac{\text{\# transformants}}{\text{µg plasmid DNA}}$$

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