One Shot® MAX Efficiency® DH5α™-T1R Competent Cells

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Kit Contents and General Information

Storage

Upon receipt, store the cells at -70°C or -80°C. Do not store in liquid nitrogen.

Contents

Each kit (20 reactions, Catalog no. 12297-016) contains the following:

Component	Composition	Amount
Chemically Competent DH5α–T1^R	--	21 × 50 µl
SOC Medium	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
pUC19	10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8	50 µl

Genotype

F$^-$ ϕ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *rec*A1 *end*A1 *hsd*R17(r_k^-, m_k^+) *pho*A *sup*E44 *thi*-1 *gyr*A96 *rel*A1 *ton*A (confers resistance to phage T1)

Quality Control Procedure

All competent cells are qualified as follows:

- 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 ~1 x 10^9 cfu/µg DNA.
- To verify the absence of phage contamination, 0.5-1 ml of competent cells are added to LB top agar and poured onto LB plates. After overnight incubation, no plaques should be detected.
- Untransformed cells are plated on LB plates 100 µg/ml ampicillin, 25 µg/ml streptomycin, 50 µg/ml kanamycin, or 15 µg/ml chloramphenicol to verify the absence of antibiotic-resistant contamination.

Reference

Killmann, H., Benz, R., and Braun, V. (1996). Properties of the FhuA channel in the *Escherichia coli* outer membrane after deletion of FhuA portions within and outside the predicted gating loop. J Bacteriol *178*, 6913-20.

Overview

Description

DH5α–T1^R Competent *E. coli* is resistant to the lytic bacteriophages T1 and T5 (Killmann *et al.*, 1996). Since bacteriophage T1 transmits easily by aerosolization, it is a hazard in high throughput laboratories and genomic centers. This strain contains a mutation in the *ton*A locus, conferring resistance to both T1 and T5 bacteriophages.

Applications

DH5α–T1^R is suitable for:

- Construction of gene banks
- Generation of cDNA libraries using plasmid-derived vectors
- Blue/white screening of transformants on selective plates containing Bluo-gal or X-gal
- High quality plasmid preparation
- Hosting M13mp cloning vectors (see page 4)

Important

One Shot® DH5α–T1^R *E. coli* **does 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.

General Handling

Be extremely gentle when working with competent cells. Competent cells are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Transformation should be started immediately following the thawing of the cells on ice. **Mix by swirling or tapping the tube gently, not by pipetting or vortexing.**

Transforming Chemically Competent Cells

Materials Supplied by the User

You will need the following items for transformation:

- 37°C shaking and non-shaking incubator
- 10 cm diameter LB agar plates with appropriate antibiotic
- Ice bucket with ice
- 42°C water bath

Before Starting

- Equilibrate a water bath to 42°C
- Warm the vial of SOC medium to room temperature
- Spread X-Gal onto LB agar plates with antibiotic, if desired
- Warm the plates in a 37°C incubator for 30 minutes
- Obtain a test tube rack that will hold all transformation tubes so that they can all be put into a 42°C water bath at once.

Procedure

The instructions provided below are for general use. Plasmid DNA should be free of phenol, ethanol, protein, and detergents for maximum transformation efficiency.

1. Briefly centrifuge the ligation reaction and place on ice.
2. Thaw, on ice, one 50 µl vial of One Shot® cells for each ligation/transformation.
3. Pipet 1 to 5 µl of each ligation reaction directly into the competent cells and mix by tapping gently. **Do not mix by pipetting up and down.** Store the remaining ligation reaction at -20°C.
4. Incubate the vial on ice for 30 minutes.
5. Incubate for exactly 30 seconds in the 42°C water bath. Do not mix or shake.
6. Remove vial from the 42°C bath and place on ice.
7. Add **250 µl** of pre-warmed SOC medium to each vial. (SOC is a rich medium; sterile technique must be practiced to avoid contamination.)
8. Place the vial in a microcentrifuge rack on its side and secure with tape to avoid loss of the vial. Shake the vial at 37°C for exactly 1 hour at 225 rpm in a shaking incubator.
9. Spread 20 µl to 200 µl from each transformation vial on separate, labeled LB agar plates. We recommend that you plate two different volumes. **Note**: You may have to dilute cells 1:10 to obtain well-spaced colonies.
10. Store the remaining transformation reaction at +4°C and plate out the next day, if desired.
11. Invert the plates and incubate at 37°C overnight.
12. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

Using DH5α-T1^R as a Transient Host

Introduction

DH5α-T1^R competent *E. coli* support replication of M13mp vectors, but it does not support plaque formation. Plating on a lawn of *E. coli* containing the F plasmid will allow plaque formation.

Before Starting

You will need the following reagents:

- Log-phase *E. coli* containing the F plasmid (*e.g.* DH5α-FT™, DH5αF′™, DH5αF′IQ™, JM101, or JM107)
- Liquid top agar containing 50 µg/ml Bluo-gal or X-gal and 1 mM IPTG
- LB plates (no antibiotic)
- SOC medium (Catalog no. 15544-018)

Procedure

A general procedure is provided below for your convenience; you may have to optimize the protocol for your particular circumstances.

1. Thaw, on ice, one 50 µl vial of One Shot® cells for each transformation.
2. Transform 10 pg of replicative form (RF) M13mp into 50 µl of competent cells.
3. Incubate the vial on ice for 30 minutes.
4. Incubate for exactly 30 seconds in the 42°C water bath. Do not mix or shake.
5. Remove vial from the 42°C bath and place on ice.

   Note: Since selection by antibiotic resistance is not necessary for plaque formation, recovery in medium is not necessary. However, it is necessary to dilute the transformation reaction 100- to 500-fold to obtain 100-300 well-spaced plaques. Dilute the transformation reaction with SOC medium and keep on ice.
6. Take the log-phase *E. coli* containing the F plasmid and add to the liquid top agar.
7. Add 30-50 µl of the diluted transformation reaction from Step 6 to the top agar.
8. Mix and pour the top agar onto LB plates (no antibiotic).
9. After the plate has solidified, invert and incubate at 37°C overnight or until plaques form.

Transforming Control Plasmid DNA

If you do not obtain the expected number of colonies, we recommend that you test the efficiency of the competent cells. Transform DH5α-T1^R with the supercoiled pUC19 plasmid supplied with the kit as described below.

1. Prepare LB agar plates containing 100 µg/ml ampicillin.
2. Transform 5 µl (50 pg) pUC19 into 50 µl of competent cells.
3. Follow Steps 4-8 on page 3.
4. Dilute the transformation reaction 1:100 and plate 30 µl on selective plates.
5. Incubate overnight at 37°C and count colonies. Calculate transformation efficiency using the formula below.

Calculation

Calculate the transformation efficiency as transformants per 1 µg of plasmid DNA.

For chemically competent cells, use the formula below to calculate transformation efficiency:

$$\frac{\text{\# of colonies}}{\text{50 pg transformed DNA}} \times \frac{10^6 \text{ pg}}{\text{µg}} \times \frac{\text{300 µl total transformation volume}}{\text{30 µl plated}} \times 100 = \frac{\text{\# transformants}}{\text{µg plasmid DNA}}$$

Expected transformation efficiency: ≥1 x 10^9 cfu/µg supercoiled plasmid

Transformation efficiencies for cDNA and ligation of inserts to vectors will be lower than for a supercoiled control plasmid such as pUC19.

- For cDNA, transformation efficiencies may be 10- to 100-fold lower.
- For ligation of inserts to vectors, transformation efficiencies may be 10-fold lower.

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...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

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