# MAX Efficiency™ DH5α™ Competent Cells

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**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

## Product description

The Invitrogen™ MAX Efficiency™ DH5α™ Competent Cells have been prepared by a patented modification of the Hanahan procedure. These cells are suitable for constructing gene banks or for generating cDNA libraries using plasmid-derived vectors. The ϕ80d*lac*ZΔM15 marker provides α-complementation of the β-galactosidase gene from pUC or similar vectors and, therefore, can be used for blue/white screening of colonies on bacterial plates that contain Bluo-Gal or X-Gal. The DH5α™ cells can be efficiently transformed with large plasmids, and can also serve as a host for the M13mp cloning vectors if a lawn of DH5α-FT™, DH5αF′™, DH5αF′IQ™, JM101, or JM107 is provided to allow plaque formation.

### 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 λ$^-$ *thi*$^-$1 *gyr*A96 *rel*A1

## Contents and storage

| Contents | Amount | Storage |
|---|---|---|
| MAX Efficiency™ DH5α™ Competent Cells | 5 × 200 µL | –80°C[1] |
| pUC19 DNA (0.01 µg/mL)[2] | 100 µL | –20°C |
| S.O.C. Medium | 2 × 6 mL | 4°C or room temperature |

[1] Do not store in liquid nitrogen.

[2] Plasmid DNA for use as a control

## Procedural guidelines

- For best results, thaw each vial of cells only once. Although the cells can be refrozen, subsequent freeze-thaw cycles will lower transformation frequencies by ~2-fold.
- Media other than S.O.C. Medium can be used, but the transformation efficiency will be reduced. Expression in Luria Broth reduces transformation efficiency a minimum of 2- to 3-fold.
- To obtain maximum transformation efficiency, the experimental DNA must be free of phenol, ethanol, protein, and detergents.
- Transformation efficiencies will be approximately 10-fold lower for ligation of inserts to vectors than for an intact control plasmid. Ligation reactions should be diluted 5-fold before using the DNA in a transformation. Only 1 µL of this dilution should be used. A standard ligation reaction (20 µL) normally contains 100–1,000 ng of DNA. Therefore, adding 1 µL of diluted DNA will result in the addition 1–10 ng of ligated DNA to the cells. We have observed that the cells begin to saturate with 10–50 ng of DNA. Also our data show that the 5-fold dilution of ligation mixtures results in more efficient transformation.
- MAX Efficiency™ DH5α™ Competent Cells can support the replication of M13mp vectors. However, DH5α™ is $F^-$ and cannot support plaque formation. Therefore, log phase DH5α-FT™, DH5αF'™, DH5αF'IQ™, JM101, or JM107 cells must be added to the top agar, which should contain X-Gal (Cat. No. 15520-034) or Bluo-Gal (Cat. No. 15519-028), final concentration 50 µg/mL, and IPTG (Cat. No. 15529-019), final concentration 1 mM. The competent cells should be added to the top agar after lawn cells, IPTG, and Bluo-Gal or X-Gal have been added. Incubation at 37°C for 1 hour is not required after adding S.O.C. Medium.

## Transform competent cells

A stock solution of pUC19 DNA (0.01 µg/mL) is provided as a control to determine the transformation efficiency. You can use the stock solution of pFastBac™ 1-Gus (0.2 ng/µL), provided with the Bac-to-Bac™ Vector Kit (Cat. No. 10360-014), as a control for the transposition frequency.

1. Thaw competent cells on wet ice. Place the required number of 17 × 100 mm polypropylene tubes on ice.

   **Note:** We recommend Samco™ 17 × 100 mm Disposable Culture Tubes (Cat. No. 17-003S) or similar.

2. Gently mix the cells, then aliquot 100 µL of the cells into the chilled polypropylene tubes.
3. Refreeze any unused cells in a dry ice/ethanol bath for 5 minutes before returning to the –70°C freezer. Do not use liquid nitrogen.

4. To determine the transformation efficiency, add 5 µL (50 pg) of the control pUC19 DNA to one tube that contains 100 µL of competent cells. Move the pipette through the cells while dispensing. Gently tap the tube to mix.
5. For DNA from ligation reactions: Dilute the reactions 5-fold in 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA. Add 1 µL of the dilution to the cells (1–10 ng of DNA), moving the pipette through the cells while dispensing. Gently tap the tubes to mix.
6. Incubate the cells on ice up to 30 minutes.
7. Heat-shock the cells for 45 seconds in a 42°C water bath; do not shake.
8. Place on ice for 2 minutes.
9. Add 0.9 mL of room-temperature S.O.C. Medium.
10. Shake at 225 rpm (37°C) for 1 hour.
11. For the control pUC19 DNA reaction: Dilute 1:100 with S.O.C. Medium. Spread 100 µL of this dilution on LB or YT plates with 100 µg/mL of ampicillin.
12. For the experimental reactions: Dilute as needed, then spread 100–200 µL of this dilution as described in step 11.
13. Incubate overnight at 37°C.

## Grow transformants

Grow MAX Efficiency™ DH5α™ Competent Cells that have been transformed with pUC-based plasmids at 37°C overnight in TB. A 100-mL growth in a 500-mL baffled shake flask will yield ~1 mg of pUC19 DNA.

## Calculate transformation efficiency

Generally, transformation efficiencies will be 10- to 100-fold lower for cDNA than for an intact control plasmid such as pUC19 DNA. ~50,000 transformants/5 ng of cDNA may be obtained. The amount of cDNA used in a 100-µL transformation should be 1–5 ng in ≤5 µL.

Calculate the transformation efficiency (CFU/µg) as follows:

$$\frac{\text{CFU in control plate}}{\text{pg of pUC19 DNA used in transformation}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \text{dilution factor(s)}$$

For example, if 50 pg of pUC19 DNA yields 100 colonies when 100 µL of a 1:100 dilution is plated, then:

$$\text{CFU/µg} = \frac{100 \text{ CFU}}{50 \text{ pg}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{1 \text{ mL}}{0.1 \text{ mL plated}} \times 10^2 = 2 \times 10^9$$

## Limited product warranty

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| Revision | Date | Description |
|---|---|---|
| A.0 | 24 May 2016 | Format, style, and legal updates |
| — | 26 October 2006 | Baseline for this revision history |



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