invitrogen™

# Library Efficiency® DH5α™ Competent Cells

**Cat. No. 18263-012**

**Size: 1 ml**
**Store at -80°C**
**(Do not store in liquid nitrogen)**

## Description

Library Efficiency® DH5α™ Competent Cells are prepared by a patented modification of the procedure of Hanahan (1). These cells are suitable for:

- Cloning experiments using limiting amounts of DNA
- Blue/white screening on X-Gal or Bluo-Gal (φ80d*lac*ZΔM15 marker)
- Efficient transformation of large plasmids
- Hosting of M13mp cloning vectors using a lawn of DH5α-FT™, DH5αF'™, DH5αF'IQ™, JM101, or JM107 for plaque formation.

| **Component** | **Amount** |
|---|---|
| DH5α™ Competent Cells | 5 x 200 µl |
| pUC19 DNA (10 pg/µl) | 50 µl |
| S.O.C. Medium | 2 x 6 ml |

## 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 $\lambda^-$

## Quality Control

Library Efficiency® DH5α™ Competent Cells are tested for a transformation efficiency of >1 x $10^8$ transformants/µg pUC19 using 50 pg of DNA and the protocol on the next page.

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## Transformation Procedure

pUC19 control DNA (10 pg/µl) is provided to check transformation efficiency. Use experimental DNA that is free of phenol, ethanol, protein, and detergents to obtain maximum transformation efficiency.

1. Thaw competent cells on wet ice. Place required number of 17 x 100 mm polypropylene tubes (Falcon® 2059; see Note 1) on ice.
2. Gently mix cells, then aliquot 100 µl competent cells into chilled polypropylene tubes.
3. Refreeze any unused cells in a dry ice/ethanol bath for 5 minutes before returning them to the -80°C freezer. **Do not use liquid nitrogen.**
4. 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 DNA), moving the pipette through the cells while dispensing. Gently tap tubes to mix.
5. (Optional) To determine transformation efficiency, add 5 µl (50 pg) control DNA to one tube containing 100 µl competent cells. Move the pipette through the cells while dispensing. Gently tap tube to mix.
6. Incubate cells on ice for 30 minutes.
7. Heat-shock cells 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. Dilute the reaction containing the control plasmid DNA 1:10 with S.O.C. Medium. Spread 100 µl of this dilution on LB or YT plates with 100 µg/ml ampicillin and 50 µg/ml X-gal (Catalog no. 15520-034) or Bluo-gal (Catalog no. 15519-028)
12. Dilute experimental reactions as necessary and spread 100-200 µl of this dilution as described in Step 11.
13. Incubate plates overnight at 37°C.

## Growth of Transformants for Plasmid Preparations

Grow DH5α™ cells that have been transformed with pUC-based plasmids overnight at 37°C overnight in TB (Catalog no. 22711-022) (2). A 100 ml culture in a 500 ml baffled shake flask will yield approximately 1 mg of pUC19 DNA.

## Notes

1. We recommend Falcon 2059 tubes or other similar 17 × 100 mm polypropylene tubes. Using microcentrifuge tubes (1.5 ml) will reduce transformation efficiency 3- to 10-fold.
2. Library Efficiency® DH5α™ Competent Cells are refreezable. Subsequent freeze-thaw cycles will reduce transformation efficiency approximately 2-fold.
3. Using media other than S.O.C. Medium will reduce transformation efficiency. Expression in Luria Broth reduces transformation efficiency 2- to 3-fold. Additional S.O.C. Medium is available from Invitrogen (Catalog no. 15544-034).

4. Library Efficiency® DH5α™ supports replication of M13mp vectors, but it is $F^-$ and does not support plaque formation. After transformation of the M13 vector, place transformed cells on ice. Add **log-phase** DH5α-FT™, DH5αF'™, DH5αF'IQ™, JM101, or JM107 cells to top agar containing 50 µg/ml X-gal or Bluo-gal, and 1 mM IPTG. Add the transformed cells to the top agar after the lawn cells, IPTG, and Bluo-gal or X-gal have been added. For a more detailed protocol, contact Technical Service (www.invitrogen.com).

5. Transformation efficiency (CFU/µg):

$$\frac{\text{CFU on control plate}}{\text{pg pUC19 DNA}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{\text{volume of transformants}}{\text{volume plated}} \times \text{dilution factor}$$

For example, if 50 pg of pUC19 yields 100 colonies when 100 µl of a 1:10 dilution is plated, then:

$$\text{CFU}/\mu\text{g} = \frac{100 \text{ CFU}}{50 \text{ pg}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{1 \text{ ml}}{0.1 \text{ ml plated}} \times 10 = 2.0 \times 10^8$$

## References

1. Hanahan, D. (1983) *J. Mol. Biol. 166*, 557.
2. Tartof, K. D. and Hobbs, C. A. (1987) *FOCUS® 9:2*, 12.

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