DH5α Competent Cells for Subcloning

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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

Thermo Scientific™ DH5α Competent Cells for Subcloning are recommended for routine subcloning into plasmid vectors. Subcloning efficiency cells are not suitable for the generation of cDNA libraries. The φ80d*lac*ZΔM15 marker provides α-complementation of the β-galactosidase gene from pUC or similar vectors to allow blue/white colony screening on bacterial agar plates containing Bluo-Gal or X-Gal.

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
DH5α Competent Cells for Subcloning	4 × 500 µL	–80°C (Do not store in liquid nitrogen)
pUC19 DNA (100 pg/µL)	20 µL	–80°C

Guidelines for tranforming cells

- For best results, thaw each vial of cells only once. Subsequent freeze-thaw cycles significantly lower transformation efficiency.
- Maximum transformation efficiency is obtained with plasmid DNA that is free of phenol, ethanol, protein, and detergents. Transformation of unpurified sample DNA or ligation reactions will result in slightly lower transformation efficiencies.
- To determine the transformation efficiency of the cells, perform a control reaction using 250 pg (2.5 µL) of the pUC19 DNA stock solution. Spread 100 µL of the pUC19 DNA control reaction on a LB plate containing 100 µg/mL of ampicillin. The cells should have a transformation efficiency of ≥1 × 10^6 cfu/µg.

Transform competent cells

1. Thaw competent cells on wet ice. Place the required number of 1.5-mL polypropylene microcentrifuge tubes on wet ice.
2. Gently mix the cells, then make 50 µL aliquots of competent cells in the chilled 1.5-mL microcentrifuge tubes.
3. Add 1–5 µL of sample DNA directly into a tube of competent cells. Mix well by gently flicking tube several times.
4. Incubate the cells on ice for 30 minutes.
5. Heat-shock the cells for exactly 20 seconds in a 42°C water bath. Do not mix or shake the tube.
6. Incubate the cells on ice for 2 minutes.
7. Add 950 µL of pre-warmed growth medium (e.g., S.O.C. or LB).
8. Place the tube on its side in a shaking incubator. Use tape to secure the tube in place.
9. Shake the tube at 225 rpm for 1 hour at 37°C.
10. Spread at least two different volumes (20–200 µL) of cells from each transformation reaction on separate LB plates containing the appropriate selective antibiotic. Label the plates with the plating volume so that the amount providing the best colony density can be identified.
11. Invert the plates and incubate overnight at 37°C.

Calculate transformation efficiency

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

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

For example, if 250 pg of pUC19 DNA yields 100 colonies when 100 µL of a 1:10 dilution is plated, then:

$$\text{CFU/µg} = \frac{100 \text{ CFU}}{250 \text{ pg}} \times \frac{1 \times 10^6 \text{pg}}{\mu\text{g}} \times \frac{1000 \text{ µL}}{100 \text{ µL plated}} \times 10 = 4 \times 10^6$$

Limited product warranty

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