Subcloning Efficiency™ DH5α™ Competent Cells

Cat. No. 18265-017 **Size: 40 reactions**
Store at -80°C

Description

Subcloning Efficiency™ DH5α™ Competent Cells are recommended for routine subcloning into plasmid vectors and are **not** suitable for the generation of cDNA libraries. The *lac*ZΔM15 marker provides α-complementation of the β-galactosidase gene, allowing blue/white screening of colonies on plates containing X-gal or Bluo-gal. DH5α™ competent cells support replication of M13mp vectors but do not support plaque formation. Plating a lawn of *E. coli* containing the F episome (*e.g.* DH5α-FT™, DH5αF'™, DH5αF'IQ™) will allow plaque formation.

Components Supplied	Amount
DH5α™ Competent Cells	4 × 500 µl
pUC19 Control DNA (100 pg/µl)	20 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 λ$^-$

Quality Control

Subcloning Efficiency™ DH5α™ Competent Cells are tested for transformation efficiency using the pUC19 control DNA supplied with the kit and using the protocol on page 2. Transformation efficiency should be greater than 1 x 10^6 transformants/µg pUC19 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity, sensitivity on nitrofurantoin (*rec*A), Lac$^-$ and Gal$^+$ phenotypes, and absence of lambda phage contamination.

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General Guidelines

Follow these guidelines when using Subcloning Efficiency™ DH5α™ competent *E. coli*.

- Handle competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting.
- Thaw 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.**
- DH5α™ cells **do not** require IPTG to induce expression from the *lac* promoter. To select transformants using blue/white screening, make sure that selective plates contain 50 µg/ml X-gal.

Transforming Competent Cells

Use this procedure to transform Subcloning Efficiency™ DH5α™ Competent Cells. We recommend verifying the transformation efficiency of the cells using the pUC19 control DNA supplied with the kit. **Do not** use these cells for electroporation.

1. Thaw on ice one tube of DH5α™ cells. Place 1.5 ml microcentrifuge tubes on wet ice.
2. Gently mix cells with the pipette tip and aliquot 50 µl of cells for each transformation into a 1.5 ml microcentrifuge tube.
3. Refreeze any unused cells in the dry ice/ethanol bath for 5 minutes before returning to the -80°C freezer. **Do not use liquid nitrogen.**
4. Add 1 to 5 µl (1-10 ng) of DNA to the cells and mix gently. **Do not mix by pipetting up and down.** For the pUC19 control, add 2.5 µl (250 pg) of DNA to the cells and mix gently.
5. Incubate tubes on ice for 30 minutes.

6. Heat shock cells for 20 seconds in a 42°C water bath without shaking.
7. Place tubes on ice for 2 minutes.
8. Add 950 µl of pre-warmed medium of choice to each tube.
9. Incubate tubes at 37°C for 1 hour at 225 rpm.
10. Spread 20 µl to 200 µl from each transformation on pre-warmed selective plates. We recommend plating two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, plate 100 µl on an LB plate containing 100 µg/ml ampicillin.
11. Store the remaining transformation reaction at +4°C. Additional cells may be plated out the next day, if desired.
12. Incubate plates overnight at 37°C.

Using DH5α™ as a Transient Host

To use the DH5α™ strain as a transient host, follow the transformation protocol provided on the previous page with the following changes:

- Since antibiotic selection is not necessary for plaque formation, recovery medium and recovery time at 37°C for 1 hour is not required.
- Add a lawn of *E. coli* containing the F episome (*e.g.* DH5α-FT™, DH5αF'™, DH5αF'IQ™) to the top agar.
- Add X-gal or Bluo-Gal to the top agar to a final concentration of 50 µg/ml and IPTG to a final concentration of 1 mM.
- Add the transformation reaction to the top agar after lawn cells, IPTG, and X-gal or Bluo-gal have been added.

Calculating Transformation Efficiency

Transformation efficiency (# transformants/µg DNA) =

$$\frac{\text{\# of colonies}}{\text{pg pUC19 DNA}} \times \frac{10^6 \text{ pg}}{\mu\text{g}} \times \frac{\text{volume of transformants}}{\text{X µl plated}} \times \frac{\text{dilution}}{\text{factor}}$$

For example, if transformation of 250 pg of pUC19 DNA yields 100 colonies when 100 µl of the transformation is plated, then the transformation efficiency is:

$$\frac{100 \text{ colonies}}{250 \text{ pg DNA}} \times \frac{10^6 \text{ pg}}{\mu\text{g}} \times \frac{1000 \text{ µl}}{100 \text{ µl plated}} \times 1 = 4.0 \times 10^6$$

Accessory Products

The following products may be used with Subcloning Efficiency™ DH5α™ Competent Cells.

Item	Amount	Catalog no.
S.O.C. Medium	10 x 10 ml	15544-034
X-gal	100 mg	15520-034
	1 g	15520-018
Bluo-gal	1 g	15519-028
IPTG	1 g	15529-019
Ampicillin	200 mg	11593-019