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I. DESCRIPTION

GenScript High-Efficiency DH5α Competent Cells are premade chemically competent cells. Their transformation efficiencies are approximately around 1×10^8 and 1×10^9 transformants/μg pUC19, which makes these competent cells optimal for cloning experiments using limiting amounts of DNA and efficient transformation of large plasmids. *LacZΔ M15* allows for α-complementation required for blue/white color selection when *E. coli* is plated on X-gal containing agar.

II. KIT CONTENTS

Product	Cat. No.	Transformation Efficiency (cfu/μg)	Format
High-Efficiency DH5α Competent Cells	M00111	1×10^8 - 1×10^9	100 μl/tube X 25 vials

III. KEY FEATURES

- ◆ Stable transformation efficiency: around 1×10^8 and 1×10^9 cfu/μg pUC19
- ◆ Blue/white screening on X-gal
- ◆ Efficient transformation of limiting-ligation reactions
- ◆ Efficient transformation of large plasmids

IV. APPLICATIONS

- ◆ Cloning experiments using limiting amounts of DNA
- ◆ Transformation of large plasmids



V. GENOTYPE

The genotype of DH5α is as follows:

F⁻, Φ80d*lacZ*Δ M15, Δ (*lacZYA-argF*) U169, *deoR*, *recA1*, *endA1*, *hsdR17* (*r_k⁻*, *m_k⁺*), *phoA*, *supE44*, λ⁻, *thi-1*, *gyrA96*, *relA1*

VI. STORAGE

The competent cells are stable and can retain their transformation efficiency for more than six months if preserved at -80°C.

VII. GENERAL PROTOCOL

A supercoiled and pure pUC19 stock (0.01 ng/μl) can be used as a control to determine the transformation efficiency of these competent cells.

1. Place the competent cells onto wet ice and thaw. Cells can also be thawed by hand, but warming above 0°C will decrease the transformation efficiency.
2. **(Optional)** To determine the transformation efficiency, add 1 μl of pUC19 (0.01 ng/μl) to one tube of competent cells. Gently tap tube and mix.
3. For DNA from ligation reactions, dilute the ligation reaction 5-fold in TE buffer, and add 1 μl of the dilution to the cells (about 1-10 ng). Gently tap tubes to mix.
4. Incubate cells on ice for 30 minutes.
5. Heat-shock cells for 90 seconds in a 42°C water bath. Do not shake.
6. Place on ice for two minutes.
7. Add 0.9 ml room-temperature SOC medium.
8. Shake at 200-230 rpm (37°C) for one hour.
9. **(Optional)** Remove 100 μl reaction containing the control plasmid DNA and spread on LB plates with 100 μg/ml ampicillin.
10. Dilute the experimental reactions as necessary and spread 100 to 200 μl of the dilution on selection plate.
11. Incubate overnight at 37°C.



VIII. NOTE

Medium other than SOC, such as LB, can also be used for transformation, but the transformation efficiency will decrease.

To determine the transformation efficiency, use supercoiled, pure pUC. Non-supercoiled or dirty plasmids will give low transformation efficiency.

Transformation efficiency (CFU/μg):

$$\frac{\text{CFU in control plate}}{\text{ng pUC19 used in transformation}} \times \frac{1 \times 10^3 \text{ ng}}{\mu\text{g}} \times \text{dilution factor}$$

For example, if 0.01ng pUC19 yields 300 colonies when 100 μl dilution is plated, then:

$$\text{CFU}/\mu\text{g} = \frac{300 \text{ CFU}}{0.01 \text{ ng}} \times \frac{1 \times 10^3 \text{ ng}}{1 \mu\text{g}} \times \frac{1 \text{ ml}}{0.1 \text{ ml}} = 3 \times 10^8$$

IX. ORDERING INFORMATION

High-Efficiency DH5α Competent Cells

Cat. No. M00111

For Research Use Only.

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