E. coli Competent Cells DH5α

Cat. No.: S2GNM03h40001 (100 µl /vial)

Description:

Genomics' DH5α are chemically competent cell, which were prepared using our proprietary process to make the cells highly efficient for immediate DNA uptake. The transformation process only takes one minute, and the steps are simplified.

Genotype:

*end*A1 *rec*A1 *rel*A1 *gyr*A96 *hsd*R17(*rk-, mk+*) *pho*A *sup*E44 *thi*-1 Δ(*lac*ZYA-*arg*F)U169 Φ80 Δ(*lac*Z)M15 F^-

Efficiency:

>5 x 10^8 cfu/µg

Application:

Suitable for cloning larger plasmids and constructing cDNA libraries, while also supporting blue-white colony screening.

Kit Contents:

Contents	S2GNM03h40001
E. coli Competent Cells DH5α	100 µl x 10 vial
Control Plasmid (pUC19)	5 µl (10^{-4} µg/µl)

Storage:

Always store Competent Cells at -80°C. Thaw on ice when ready for use. Do not refreeze thawed, unused aliquots.

Protocol:

Before Starting

a. Turn on the water bath and set at 42°C.

b. LB plates containing appropriate antibiotics, 0.1mM IPTG and 40µl/ml X-Gal (or spreading 40µl of 20mg/ml X-Gal and 4µl of 200mg/ml IPTG onto LB/antibiotic plates). Solution and

media containing antibiotic, IPTG, and X-Gal must be stored protected from light in order to maintain potency.

c. Chill sterile polypropylene culture tubes and pipette tips on ice or at -20°C

Starting

<Heat-shock cold plate>

1. Thaw one tube of competent cells (Typically 100µl) on ice until 1/3~1/2 volume is thawed.
2. Add pre-chilled DNA (the volume of DNA should be ≦ 5% of competent cells) immediately. Mix by vortex for 1 second or tap the tube the tube with figure to mix well.
3. Keep the tubes on ice for 1-5 minutes to increase the transformation efficiency - ***Optional step***.
4. Heat-shock the tube in a water bath at exactly 42°C for 15~45 seconds.
5. Place the cell using Plating Beads onto a pre-chilled (4°C) and dried selective LB agar plate (LB+ antibiotics).
6. Incubate the plate at 37°C for 12-16 hours for Genomics' DH5α Competent Cells.

<Non Heat-shock cold plate>

1. Prewarm a selective LB agar plate at room temperature upto 37 °C incubator.
2. Thaw one tube of competent cells (Typically 100µl) on ice until 1/3~1/2 volume is thawed.
3. Add pre-chilled DNA (the volume of DNA should be ≦ 5% of competent cells) immediately. Mix by Vortex for 1 second or tap the tube the tube with figure to mix well.
4. Keep the tubes on ice for 1-5 minutes to increase the transformation efficiency - ***Optional step.***
5. Place the cell using Plating Beads onto a pre-warmed and dried selective LB agar plate (LB + antibiotics).
6. Incubate the plate at 37°C for 12-16 hours for Genomics' DH5α Competent Cells.

Composition of Buffers and Solutions:

LB medium with antibiotic

To 1L of distilled water, add:

10g Bacto® -Tryptone/5g Bacto® -Yeast Extract/10g NaCl. Adjust the pH to 7.0-7.5 with NaOH. Autoclave and allow the autoclaved medium to cool to 55°C and add appropriate antibiotic. For LB plates, include 15g agar prior to autoclaving.

Calculation of Transformation Efficiency (Colony Forming Units [cfu]):

Transformation efficiency is defined as the number of colony forming units (cfu) produced by 1µg of Competent Cells Control DNA (supercoiled plasmid DNA) and is measured by performing a control transformation reaction using a known quantity of DNA, typically 0.1ng, then calculation the number of cfu formed per microgram DNA.

Equation for Transformation Efficiency (cfu/µg)

$$\frac{\text{Cfu on control}}{\text{ng of Competent Cells Control DNA plated}} \times \frac{1\times 10^{3}\text{ng}}{\mu\text{g}}$$

Note: Transformation with ligated plasmid DNA will produce fewer colonies than transformation with supercoiled plasmid DNA.

◎ **Revision History** ◎

Description	Version	Date
Initial Release	GN-CPC-053_Protocol_V1	May 2021
Catalog Number Adjustment	S2GNM03h40001_Protocol_V2	Jan 2025