

GC5™ Competent Cells

Cat. No.	Size	Amount	pUC19 control (10 pg/μL)
42-652	1ml	20 x 50 μl	1 x 50 μl

Store at -70°C. FOR RESEARCH USE ONLY

General Description

GC5 Competent Cells are chemically competent *E. coli* cells. The cells are transformed by heat-shock methods. These competent cells are resistant to the lytic bacteriophages T1 and T5.

GC5 Competent Cells are suitable for many molecular biology applications, like generating cDNA libraries from plasmid-based vectors or constructing gene banks. Blue/white screening for recombinants can be performed by including X-gal and IPTG in the agar plates.

The GC5 strain is sensitive to these common antibiotics: ampicillin, kanamycin, chloramphenicol and tetracycline. It is resistant to nalidixic acid.

General Handling

- Competent cells are very sensitive to any change in temperature. Cells must be thawed on ice. The transformation should be started immediately after the cells are thawed.
- Competent cells must be treated gently. Mix cells by swirling or gently tapping the reaction tube. Do not mix by pipetting or vortexing.
- Once thawed, the cells should be used. Re-freezing thawed competent cells will result in a significant drop in transformation efficiency.

Genotype

F⁻ φ80/lacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1
hsdR17(rk⁻, mk⁻) phoA supE44 thi-1 gyrA96 relA1 λ⁻ tonA

Efficiency

≥10⁸ - 10⁹ transformants/μg pUC19 DNA

pUC19 DNA Storage Buffer

Control DNA is supplied in TE Buffer [10mM Tris-HCl pH 8.0, 0.1mM EDTA].

Notes on Ligation Reactions

Ligation reactions inhibit transformation. Less transformants are observed from ligation reactions than from transformations with plasmid DNA.

Use 0.5 μL of a ligation reaction per 50μL of competent cells. For best results, either purify the ligation mixture by ethanol precipitation prior to transformation or dilute the ligation reaction 3-fold in TE buffer and use 1 μL per 50 μL competent cells.

Advance Preparations

- Equilibrate a non-shaking water bath to 42°C.
- Place SOC Medium at room temperature.
- Prepare LB agar plates with the appropriate antibiotic. If blue/white screening for recombinants is desired, the plates should include 40μg/mL X-gal and 1mM IPTG.
- Agar plates should be placed in a 37°C incubator for about 30min. prior to plating.

Transformation Protocol for Chemically Competent GC5 Cells

- Remove competent cells from -70°C and place directly in ice. Thaw cells for 5 to 10 min.
- Gently mix cells by tapping tube.
- Add 1-50 ng of DNA [or 1 μL control DNA] into the 50 μL competent cells. Swirl the pipettor tip through the cells while dispensing DNA. Gently tap tube to mix.
- Place the tubes on ice for 30 min.
- Heat-shock the cells for 45 sec. in a 42°C water bath. Do not shake.
- Add 450 μL of room temperature SOC medium to each transformation reaction.
- Incubate at 37°C for one hour, with shaking (225 to 250 rpm).
- Spread on LB agar plates containing appropriate antibiotic (e.g., 100 μg/mL ampicillin for control pUC19).
- Incubate the plates at 37°C overnight (12 to 16 hours).

SOC Medium Formulation

2% Tryptone, 0.5% Yeast Extract, 0.4% glucose, 10mM NaCl, 2.5mM KCl, 5mM MgCl₂, 5 mM MgSO₄.

Quality Control

Cells must have a transformation efficiency of $\geq 10^8$ - 10^9 transformants/ μ g pUC19 DNA (non-saturating conditions). Cells must show resistance to T5 phage.



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