GENE CHOICE
A Genesee Scientific Brand

GC5™ Competent Cells

Cat. No.	Size	Amount	pUC19 control (10 pg/µL)
42-650	2.0 mL	10 x 200 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.

Value Efficiency GC5 Competent Cells are used for routine subcloning and other applications in which maximum transformation efficiency is not required. These cells are still very competent and give excellent results even for cloning experiments. 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 naladixic 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*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$^-$ *ton*A

Efficiency

≥10^8 - 10^9 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 0.5µL 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 1µL per 50µL 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

1. Label and place sterile 17 x 100mm polypropylene tubes (Falcon® 2059) on ice, one tube per transformation.
2. Remove competent cells from -70°C and place directly in ice. Thaw cells for 5 to 1 min.
3. **Gently mix cells by tapping tube**, then transfer 50µL of cells into chilled tubes.
4. Add 1 to 50 ng of DNA [or 1µL control DNA] per 50µL of cells. Swirl the pipettor tip through the cells while dispensing DNA. Gently tap tube to mix.
5. Place the tubes on ice for 30min.
6. Heat-shock the cells for 45sec. in a 42°C water bath. Do not shake.
7. Add 450µL of room temperature LB, YT or SOC medium to each transformation reaction.
8. Incubate at 37°C for one hour, with shaking (225 to 250rpm).
9. Spread on LB agar plates containing appropriate antibiotic (*e.g.*, 100µg/mL ampicillin for control pUC19).
10. 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 $MgCl_2$, 5mM $MgSO_4$.

Quality Control

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

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