GC10™ Competent Cells

Cat. No.	Size	Amount	pUC19 control (10 pg/µL)
42-657	0.5ml	10 x 50µl	1 x 50µl
42-658	1ml	20 x 50µl	1 x 50µl
42-659	3.75ml	75 x 50µl	1 x 50µl
42-660	1ml	5 x 200µl	1 x 50µl
42-661	15ml	75 x 200µl	1 x 50µl

Store at -7**0°C**. FOR RESEARCH USE ONLY

General Description

GC10 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.

GC10 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 GC10 strain is sensitive to these common antibiotics: ampicillin, kanamycin, chloramphenicol and tetracycline. It is resistant to streptomycin.

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- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80d*lac*ZΔM15 Δ*lac*X74 *end*A1 *rec*A1 Δ(*ara*, *leu*)7697 *ara*D139 *gal*U *gal*K *nup*G *rps*L λ- T1R

Efficiency

≥ 10^8-10^9 transformants/µg of 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 30 min. prior to plating.

Transformation Protocol for Chemically Competent GC10 Cells

1. Remove competent cells from -70°C and place directly in ice. Thaw cells for 5 to 10min.
2. **Gently mix cells by tapping tube**
3. Add 1 to 50ng 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.
4. Place the tubes on ice for 30min.
5. Heat-shock the cells for 45sec. in a 42°C water bath. Do not shake.
6. Add 450µL of room temperature SOC medium to each transformation reaction.
7. Incubate at 37°C for one hour, with shaking (225 to 250rpm).
8. Spread on LB agar plates containing appropriate antibiotic (*e.g.*, 100µg/mL ampicillin for control pUC19).
9. 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 $MgCl_2$, 5 mM $MgSO_4$.

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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