

Product Information

GC5™ Competent Cells, Uni Pack

Catalog Number **G3169**

Storage Temperature $-70\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

Product Description

GC5™ Competent Cells are a high transformation efficiency *E. coli* strain that is comparable to DH5α™. GC5 chemically competent cells are suitable for molecular biology applications such as plasmid propagation, cDNA library generation from plasmid-based vectors, and general cloning protocols. This K strain bacterium has *recA1* and *endA1* mutations that increase plasmid stability and improve the quality of plasmid preparations, respectively. GC5s carry the *lacZΔM15* mutation for blue/white screening of recombinants via α-complementation with the amino terminus of β-galactosidase. GC5 competent cells are also resistant to the lytic bacteriophages T1 and T5, to protect samples from phage contamination.

Sigma's GC5 competent *E. coli* cells are grown and made chemically competent using an optimized procedure specific to the strain, followed by strain verification and efficiency testing. The cells are provided in frozen 50 μL aliquots for convenience. Each aliquot may be used for a single transformation. The cells are guaranteed to have a transformation efficiency of $>1 \times 10^9$ cfu/μg when transformed with non-saturating amounts of pUC19 Control DNA.

Genotype of GC5:

$F^- \Phi 80/lacZ\Delta M15 \Delta(lacZYA-argF)U169\ endA1\ recA1\ relA1\ gyrA96\ hsdR17\ (r_k^-, m_k^+)\ phoA\ supE44\ thi-1\ \lambda^-$
T1R

Components/Reagents

GC5 Competent Cells are packaged with sufficient reagents for 10 or 20 transformation reactions.

- GC5 Chemically Competent Cells, 10 x 50 μL or 20 x 50 μL, Catalog Number G2669
- pUC19 Control DNA, 10 ng/μL, 10 μL, Catalog Number D2567

Reagents and Equipment Required but Not Provided

(Sigma Product Numbers have been given where appropriate)

- Shaker incubator (37 °C)
- Cabinet incubator (37 °C)
- Heated water bath (37 °C)
- SOC Medium, Catalog No. S1797
- LB Agar EZMix™ Powder, Catalog No. L7533
- Selection antibiotic
- 15 ml polypropylene culture tubes (sterile)
- Culture dishes
- Sterile Spreaders, Catalog No. Z376779

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

All components are guaranteed to be stable for at least six months after receipt when stored at $-70\text{ }^{\circ}\text{C}$.

Procedure

Handling Tips:

1. Verify that the cells are still frozen and dry ice is still present in the shipping container upon receipt.
2. Handle the tubes as little as possible to prevent accidental warming of the cells. Keep cells on ice at all times.
3. When mixing the cells, gently swirl or tap the reaction tube. Do not mix by pipetting or vortexing.
4. Cells can be refrozen on dry ice and returned to $-70\text{ }^{\circ}\text{C}$, however, transformation efficiency will decrease significantly with each freeze-thaw cycle.

Before Starting:

1. Prepare LB agar plates with the appropriate antibiotic for selection of colonies that contain plasmid DNA, and warm to 37 °C. If blue/white screening for recombinants is desired, the plates should include 40 µg/ml X-gal, Catalog Number B9146, or 300 µg/ml S-Gal™, Catalog Number S9811, + 500 µg/ml Ferric Ammonium Citrate, Catalog Number F5879, and 1 mM IPTG, Catalog Number I6758.
2. Heat a water bath to 37 °C.

Note: Alternatively, the water bath may be set to 42 °C, but this variation on the procedure has been shown to be slightly less robust than 37 °C.

3. Warm SOC medium to room temperature (20-25 °C).

I. Standard Transformation Protocol In Brief

Note: See following section for detailed protocol

1. Thaw the required number of tubes containing cells on wet ice.
2. Add 1–50 ng of DNA (or 1 µL of control DNA) to cells and gently tap the tube to mix.
3. Place cells on wet ice for 30 minutes.
4. Heat shock cells by incubating tubes in a 37 °C water bath for **exactly** 45 seconds.
5. Return the cells to ice for 2 minutes.
6. Add 450 µL of SOC, at room temperature, to each transformation reaction.
7. Shake at 225 rpm at 37 °C for 1 hour.
8. Use a sterile spreader to evenly distribute transformation reaction over LB agar plates containing the appropriate selection antibiotic.
9. Incubate plates overnight at 37 °C.

II. Detailed Standard Transformation Protocol

Note: DNA in ligation reactions containing high quality reagents can be added directly to Sigma's competent cells. Inactivation of the ligase is not required prior to transformation. No more than 50 ng of ligation reaction should be used in a 50 µL transformation reaction.

Plasmid DNA isolated using miniprep procedures is typically satisfactory. To achieve maximum efficiency, the sample DNA should be free of phenol, ethanol, salts, protein, and detergents, and be dissolved in TE buffer or water.

1. Remove the required tubes of cells from the –70 °C freezer, including one extra for the control DNA if desired. Place tubes immediately on wet ice so that only the cap is visible above the ice. Allow the cells to thaw on ice for approximately 5 minutes.
2. Visually examine cells to ensure they are thawed, and gently tap the vial several times to resuspend cells.
3. (Optional) Add 1 µL pUC19 Control DNA to one tube of cells. Mix gently by tapping the tube. Return the cells to the ice.
4. Add 1 ng to 50 ng ligation reaction or purified plasmid DNA directly to cells. Mix as in #3.
5. Incubate the cells on wet ice for 30 minutes.
6. Heat shock the cells by incubating tubes in a 37 °C water bath for **exactly** 45 seconds.
7. Replace the cells on ice immediately for at least 2 minutes.
8. Add 450 µL of SOC medium, at room temperature, into each tube containing the cell/DNA mixture. Optimal recovery may be achieved by transferring the cells to a sterile 15 ml polypropylene culture tube. The cap should be loose to ensure sufficient air exchange and aeration of the culture.
9. Incubate cells at 37 °C with shaking (225-250 rpm) for 1 hour.

- Pipette 10-100 μL of each transformed cell suspension onto LB agar plates containing selection antibiotic and evenly distribute using a sterile spreader. Plates should be pre-warmed to 37 °C for optimal colony growth. When inoculating less than 25 μL of cell suspension, first pipette a drop of SOC onto the plate and add cell suspension to the SOC.

Note: The amount of transformation mixture to plate varies with the efficiency of both the ligation and the competent cells. When using the control DNA, add no more than 10 μL into a drop of SOC on an LB agar plate containing 100 $\mu\text{g}/\text{ml}$ ampicillin or carbenicillin.

III Rapid Transformation Procedure

A. Notes

- Rapid transformation may be used with high quality plasmid DNA.
- Although considerable time (up to 60 minutes) may be saved following the rapid transformation protocol, a loss of 10- to 100-fold transformation efficiency should be expected.
- Follow the previous Handling Tips and Before Starting Tips, pages 1 and 2.
- When using kanamycin for the selection antibiotic, the standard transformation protocol is recommended.

B. Rapid Procedure

- Remove the required tubes of cells from the $-70\text{ }^{\circ}\text{C}$ freezer, including one extra for the control DNA if desired. Place tubes immediately on wet ice, so that only the cap is visible above the ice. Allow the cells to thaw on ice for approximately 5 minutes.
- Visually examine cells to ensure they are thawed, and gently tap the vial several times to resuspend cells.
- (Optional) Add 1 μL pUC19 Control DNA to one tube. Mix gently by tapping the tube. Return the tube to the ice.

- Add 1-50 ng of ligation reaction to the cells. Mix as in step #3 and incubate on ice 3-5 min.
- Heat shock the cells by incubating in a 37 °C water bath for **exactly** 45 sec.
- Immediately replace the cells on ice and incubate for 2 minutes.
- Add 200 μL of SOC, at room temperature, to the cells.
- Pipette 20-200 μL of each transformed cell suspension onto LB agar plates containing the selection antibiotic and evenly distribute using a sterile spreader. Plates should be pre-warmed to 37 °C for optimal colony growth. When inoculating with less than 25 μL of cells, first pipet a drop of SOC onto the plate and add cell suspension to the SOC.

Note: The appropriate amount of transformation mixture to plate varies with the efficiency of both the ligation reaction and the competent cells. When using the control DNA, add no more than 10 μL of the cell suspension to a drop of SOC on an LB agar plate containing 100 $\mu\text{g}/\text{ml}$ ampicillin or carbenicillin.

- Incubate plates overnight at 37 °C.

References

- Boyer, H. W., and Roulland-Dussoix, D., *J. Mol. Biol.*, **41**, 459-472, (1969).
- Bolivar, F., and Backman, K., *Methods Enzymol.* **68**, 245-267 (1979).

Troubleshooting Guide

Problem	Cause	Solution	
No colonies on plate	Cells thawed and re-frozen due to improper handling	Obtain new product	
	No plasmid DNA added	Ensure plasmid DNA was added to cells	
	Plasmid DNA missing antibiotic resistance ORF and/or replication of origin	Review cloning strategy	
	Low ligation efficiency	Increase volume of transformation reaction for inoculation	
	Too much, or wrong antibiotic used	Adjust antibiotic levels in plates, Test a plate by streaking with an antibiotic resistant culture	
	Low transformation efficiency		Check to make sure transformation protocol was followed exactly
			Increase volume of transformation reaction for inoculation Check efficiency using non-saturating amounts of pUC19 Control DNA
Too many colonies on plate or a lawn of growth is observed	High transformation efficiency or high amounts of added DNA	Decrease volume of transformation reaction for inoculation	
	Too little antibiotic	Adjust antibiotic levels in plates	
	Expired antibiotic	Use fresh antibiotic in plates	
	Contaminated SOC medium	Obtain fresh medium and maintain sterile technique	
Unexpected growth on plate (i.e., fungal or mold)	Contaminated plates	Prepare and spread plates in laminar flow hood to reduce contamination	
	Contaminated SOC medium	Obtain fresh medium and maintain sterile technique	

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