

Protocol • Chemically Competent Cells • Catalog Nos. CC001/CC002, CC003/CC004

Efficient transformation, stable inserts and high-quality plasmid DNA

Description

GeneCopoeia chemically competent cells provide high-efficiency transformation and are suitable for many standard cloning applications. The cells enable fast screening with blue/white selection, ensure high-quality plasmid preparations and contain *recA1 endA1* mutations to increase insert stability.

Available in single tube format, GeneCopoeia competent cells deliver a reliable platform for producing plasmid DNA.

GeneCopoeia chemically competent cells GCI-5α and GCI-L3 are comparable to DH5α™ and Stbl3™ respectively.

Strain	GCI-5α	GCI-L3
Applications	Transformation of standard (non-lentiviral) plasmid constructs	Transformation of lentiviral plasmid constructs
Catalog numbers	CC001 (10 tubes) CC002 (20 tubes)	CC003 (10 tubes) CC004 (20 tubes)
Transformation efficiency	1 - 3 x 10 ⁹ cfu/μg pUC19 DNA	>1×10 ⁸ cfu/μg pUC19
Size	100 μl/tube	100 μl/tube
Shipping and storage conditions	Ship on dry ice and store immediately at –80 °C	Ship on dry ice and store immediately at –80 °C

Storage of plasmid samples

Plasmid DNA samples in TE buffer (pH 8.0) should be stored at 4°C.

Transformation protocol

- 1. Thaw competent cells on ice.** Place required number of polypropylene tubes (Falcon® 2059) on ice for transformation.

Note: DO NOT thaw competent cells by warming the tube between hands.
To maintain the competency, cells should be kept on ice at all times.
For best results, do not re-freeze the cells. Discard unused cells.

- 2. Mix cells gently. Transfer 100 μl of competent cells into chilled polypropylene tubes.**
- 3. Add 100-200 ng of plasmid DNA to the thawed competent cells.** Tap the tubes gently to mix DNA with cells. Do not mix by pipetting.
Note: For DNA from a ligation reaction, up to 10 μl of ligation reaction mixture can be directly added to 100 μl of competent cells. The transformation efficiency of ligated DNA will be approximately 10-fold lower than that of a supercoiled plasmid.

- 4. Incubate DNA with cells on ice for 30 minutes.** Tap the tubes every 10 minutes.

5. **Heat shock cells by placing the tubes into a 42°C water bath for 45 seconds.** Alternatively, heat shock cells for 60 seconds at 37°C. Do not shake the tubes during heat shock.
Note: Both the heat shock temperature and timing are important.
6. **Immediately place the tubes on ice.** Incubate for 2 to 5 minutes.
7. **Add 500 µl of pre-warmed SOC medium to each tube.** Invert the tubes 2 to 3 times gently to suspend cells.
Note: Media other than SOC can be used. However, the transformation efficiency will be reduced. Expression in Luria Broth reduces the transformation efficiency at a minimum of 2 to 3-fold.
8. **Incubate the tubes for one hour in a 37°C water bath.** Alternatively, incubate the tubes for one hour in a 37°C shaker at 100 to 200 rpm.
9. **Spread different aliquots of transformation culture (50 to 200 µl) on pre-warmed antibiotic-containing plates.** Store the remaining transformation mixture at 4°C for possible subsequent plating.
10. **Incubate plates for 12 to 16 hr at 37°C.**

Equipment/reagents required but not supplied

Water bath
Incubator
Shaker (optional)
Selective LB plates
SOC medium

Recipe for SOC medium

0.5% yeast extract
2% tryptone
10 mM NaCl
2.5 mM KCl
10 mM MgCl₂
10 mM MgSO₄

Autoclave the medium. Cool the medium to room temperature and add 1/100 of 2.0 M sterile-filtered glucose (final concentration 20 mM). The medium is stable for years at room temperature if kept sterile.

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