FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Biomiga, Inc.
DH5α Chemically Competent Cells

Catalog Number	CC1101
Kit Size	20 x 50 µL reactions
Version	Rev. A
Storage	−80°C

Ordering Information

Catalog Number	Description	Format
CC1101	DH5α Chemically Competent Cells	20 x 50 µL tubes

1. Intended Use

The Biomiga DH5α Chemically Competent Cells (Cat# CC1101) are intended for use in routine bacterial transformation procedures for molecular biology research applications, including plasmid propagation, subcloning, and library construction. This product is FOR RESEARCH USE ONLY and is not intended for use in diagnostic or therapeutic procedures.

2. Summary

Chemically competent cells are bacterial cells that have been treated with divalent cations (e.g., $CaCl_2$) to render their membranes transiently permeable to exogenous DNA. Upon heat shock, plasmid DNA or ligation products are taken up by the cells and subsequently propagated under antibiotic selection.

The DH5α strain is widely used for routine cloning and subcloning due to its high transformation efficiency, blue/white colony screening capability (via lacZ complementation), and low recombination frequency. DH5α cells are suitable for amplification of standard plasmids and ligation products.

3. Strain Information

Strain	E. coli DH5α
Genotype	F− Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17(rk−, mk+) phoA supE44 thi-1 gyrA96 relA1 λ−
Transformation Efficiency	>1 x 10^7 cfu/µg pUC19
Cell Density	1–2 x 10^9 bacteria/mL

4. Kit Contents

Component	Quantity	Storage
DH5α Chemically Competent Cells	20 x 50 µL	−80°C
SOC Medium	1 x 1.5 mL	−20°C or 4°C

5. Materials Required but Not Supplied

- 37°C shaking incubator
- 37°C non-shaking incubator
- 42°C water bath
- Ice bucket with ice
- LB agar plates (10-cm diameter) with appropriate antibiotic
- Microcentrifuge tubes
- Pipettes and sterile tips
- Timer

6. Before You Begin

- Equilibrate a water bath to exactly 42°C. Accurate temperature is critical for optimal heat shock efficiency.
- Pre-warm SOC medium to 37°C.
- Pre-warm LB agar plates at 37°C for at least 30 minutes before plating.
- Keep cells on ice at all times except during heat shock.
- Thaw competent cells on ice immediately before use. Do not allow to warm to room temperature.

7. Transformation Procedure

1. Remove one 50 µL aliquot of DH5α Chemically Competent Cells from −80°C and thaw on ice for 5–10 minutes.
2. Add 1–2 µL of plasmid DNA or ligation product directly to the competent cells. Mix gently by tapping or swirling. Do not vortex.
3. Incubate on ice for 20–30 minutes.
4. Heat shock the sample in a 42°C water bath for exactly 45 seconds. Do not exceed 45 seconds.
5. Immediately return the tube to ice and incubate for 2 minutes.
6. Add 250 µL of pre-warmed SOC medium (37°C). Cap and incubate at 37°C on a shaking platform (200–250 rpm) for 1 hour.
7. Plate 20–200 µL of the transformed culture onto LB agar plates containing the appropriate selection antibiotic.
8. Incubate plates at 37°C for 12–15 hours until colonies appear.

8. Quality Control

Each lot of DH5α Chemically Competent Cells is tested for transformation efficiency using pUC19 plasmid (Ampicillin resistance). Lot release criteria:

Parameter	Specification
Transformation efficiency	>1 x 10^7 cfu/µg pUC19
Cell density	1–2 x 10^9 bacteria/mL
Sterility	No growth on LB agar without plasmid

9. Storage and Stability

- Store competent cells at −80°C immediately upon receipt.
- SOC medium may be stored at −20°C (long-term) or 4°C (short-term, up to 6 months).
- Do not store competent cells at −20°C. Storage above −80°C will significantly reduce transformation efficiency.
- Cells are stable for 12 months from the date of manufacture when stored as directed.

- Each aliquot is single-use. Refreezing unused cells is not recommended.

Note: If cells were not stored at −80°C or if transformation efficiency is suspected to have decreased, confirm efficiency using pUC19 control plasmid before use in critical experiments.

10. Limitations

- These cells are optimized for transformation of standard high-copy plasmids (≤15 kb). Large constructs (>15 kb) or low-copy plasmids may yield reduced efficiency.
- DH5α cells are not suitable for propagating plasmids that require dcm− or dam− hosts.
- These cells are not recommended for expression of recombinant proteins; use expression-optimized strains (e.g., BL21) for that purpose.
- Blue/white colony screening requires X-gal and IPTG supplementation; these reagents are not included.
- Transformation efficiency may be reduced by impure DNA. Use only high-quality, purified plasmid or ligation product.

11. Warnings and Precautions

- FOR RESEARCH USE ONLY. Not for use in diagnostic or therapeutic procedures.
- Handle all biological materials in accordance with institutional biosafety guidelines.
- DH5α is a Biosafety Level 1 (BSL-1) organism. Follow standard laboratory safety practices.
- Dispose of all biological waste in accordance with applicable local, state, and federal regulations.
- Do not pipette by mouth.
- Wear appropriate personal protective equipment (gloves, lab coat, eye protection) when handling cells.

12. Troubleshooting

Problem	Possible Cause	Recommended Action
No colonies	Cells stored incorrectly; heat shock too long/short; no antibiotic resistance in insert	Verify storage conditions; confirm heat shock at exactly 42°C for 45 sec; test with pUC19 control
Low number of colonies	Impure or degraded DNA; reduced cell viability	Use freshly prepared, high-purity DNA; ensure cells were not subjected to freeze-thaw cycles
High background (no insert)	Incomplete digestion or ligation; uncut vector carryover	Verify restriction digest and ligation; gel purify vector prior to ligation
Colonies present without selection	Antibiotic degraded or omitted from plates	Prepare fresh antibiotic plates; add antibiotic after media cools to <55°C

13. Performance Characteristics

Transformation efficiency is measured by transforming 50 pg of uncut pUC19 plasmid (2,686 bp) under the protocol conditions described in this document. Results are expressed as colony-forming units per microgram of plasmid DNA (cfu/µg).

- Minimum guaranteed efficiency: >1 x 10^7 cfu/µg pUC19
- Typical efficiency: 1–5 x 10^7 cfu/µg pUC19

14. Symbols

Symbol	Description
RUO	For Research Use Only
LOT	Lot Number
IVD	In Vitro Diagnostic Medical Device (not applicable to this RUO product)

15. Manufacturer

Manufacturer	Biomiga, Inc.
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16. Revision History

Revision	Date	Description
Rev. A	2026-06	Initial release