MC1061 Chemically Competent *Escherichia coli*

Product Information Sheet
 # VS-ELS10710-01

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SUMMARY

shipped on dry ice; store at -80 °C

For research use only

Product
Chemically competent *Escherichia coli* cells of strain MC1061.

Description
The chemically competent *Escherichia coli* cells of strain MC1061 are prepared for heat shock transformation. MC1061 is a recombinant positive strain (*recA*$^+$) provided for cloning and amplification of plasmid DNA of diverse Gram-positive bacteria, e.g., plasmids for expression in *Lactococcus lactis* or *Bacillus subtilis*.

Genotype
araD139, Δ(*ara*, *leu*)*7697*, Δ*lacX74*, *galU*-, *galK*-, *hsr*-, *hsm*+, *strA* (Casadaban MJ and Cohen SN, 1980; Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*.)

Storage and Handling
Competent cells should be stored at -80 °C. Storage at -20 °C will result in a significant decrease in transformation efficiency. Care must be taken not to interrupt the cooling chain since any storage above -80 °C will lead to a loss in transformation efficiency, even if cells do not thaw.

For handling please also consider the handbook or data sheet of the plasmid or expression vector that will be transformed.

Transformation Efficiency
1-3 x 10^6 cfu/µg plasmid DNA (tested with vector pNZ2103 of *Lactococcus lactis* Constitutive Expression System, #VS-ELV01200)

Product Contents
5 x 100 µl chemically competent MC1061 *E. coli* cells in transformation buffer containing 10.5% glycerol.

Transformation Protocol
1. Thaw a tube of competent MC1061 cells on ice for 10 min.
2. Aliquot 50 µl of the competent cells to a new tube (tube 2: negative control)
3. Add 1-5 µl containing 50-100 ng plasmid DNA to the cells in the original tube (tube 1: plasmid transformation) and 1-5 µl sterile H_2O_{dd} to the cells in the control tube (tube 2).
4. Carefully mix by flicking the tubes 4-5 times. Do not vortex.
5. Place the DNA/cell mixture (tube 1) and the negative control (tube 2) on ice for 30 min.
6. Heat shock the DNA/cell mixture and the control tube at exactly 42 °C for exactly 45 sec. Do not mix!
7. Place on ice for 5 min.

8. Pipette 950 µl of SOC medium into each tube and shake them vigorously (250 rpm) at 37 °C for 60 min or rotate (Note: SOC gives 2-fold higher transformation efficiency than LB medium).
9. Prepare several dilutions (e.g., 1:10, 1:20, and 1:100) of the transformed cells (tube 1) and plate 50-100 µl of each dilution on an LB agar plate containing an antibiotic for selection. Plate 100 µl of the control preparation (tube 2) on an LB agar plate containing the same antibiotic. The kind of antibiotic is depending on the resistance gene of the transformed plasmid.
10. Incubate 1-2 days at 37 °C.

SOB medium (1 liter)
20 g Tryptone
5 g Yeast extract
0.5 g NaCl
950 ml H_2O_{dd}

shake until all is dissolved
add 10 ml of 250 mM KCl
adjust to pH 7.0 with 5 M NaOH
add H_2O_{dd} to 1 liter and autoclave

Just before use add 5 ml of a sterile 2 M $MgCl_2$ solution

SOC medium (1 liter)
980 ml SOB medium (sterile)
20 ml 1 M glucose (sterilized by filtration)

Quality Warranty

Transformation efficiency (1-3 x 10^6 cfu/µg plasmid DNA) of the chemically competent MC1061 *E. coli* cells has been tested and verified. Untransformed cells have been controlled to be sensitive to diverse antibiotics: ampicillin (100 µg/ml), chloramphenicol (10 µg/ml), and kanamycin (50 µg/ml).

Order Information, Shipping and Storage

Order#	Product	Amount
VS-ELS10710-01	MC1061 Chemically Competent *Escherichia coli*	5 x 100 µl
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