BIO-RAD

C-Max™ 5α Competent Cells 170-3342

10 x 0.05 ml

For Research use only
Store at -70°C

Storage and Stability

Store the C-Max5α Chemi-Competent Cells at -70°C in a constant temperature freezer. When stored under these conditions, the cells are stable for six months after date of receipt.

Kit Contents

C-Max™ 5α Chemi-Competent Cells are packaged in single use volumes for convenience and maintenance of efficiency. The recommended transformation protocol will yield 10 x 50 µl transformations.

C-Max5α Chemi-Competent Cells are suitable for transformation by "heat shock" at 42°C. These cells are endonuclease deficient, and well-suited for use in constructing cDNA libraries and other demanding cloning applications. C-Max5α cells carry the ϕ80d*lac*ZΔM15 marker and thus, can be used for blue-white screening when transformed with vectors carrying the β-galactosidase gene via α-complementation.

Genotype

F^-ϕ80d*lac*ZΔM15 Δ(*lac*ZYA - *arg*F)U169 *rec*A1 *end*A1 *hsd*R17(r_k^-, m_k^+) *pho*A *sup*E44 λ^- *thi*-1 *gyr*A96 *rel*A1

Reagent	Volume	Description
C-Max5α Cells	10 x 0.05 ml	Blue Cap
pUC19 Control Plasmid	1 x 0.05 ml	Yellow Cap

Quality Control

Each batch of C-Max5α F' cells is performance tested to ensure yields of >1 x 10^9 CFU/µg DNA using non-saturating amounts (20 pg) of pUC19 control plasmid.

Protocol Notes

1. Thaw cells on wet ice. For best results, use within 15 min of thawing.
2. Although ligation reactions may be diluted prior to addition to cells, ethanol precipitation of the ligation mix will give better results.

Transformation Protocol

1. Thaw one 50 µl vial of competent cells for each transformation on ice. Gently mix the cells by tapping the vial.
2. For determining the transformation efficiency, add 2 µl (20 pg) of pUC19 control DNA to one vial of competent cells. Gently mix by tapping the tube.
3. For experimental DNA, add 1 to 10 ng DNA (in ≤ 5 µl). Gently mix by tapping the tube. A stock solution of control pUC19 DNA (10 pg/µl) is supplied to determine transformation efficiency. For best results, the experimental DNA should be free of protein, detergents, organic solvents and salts. Once thawed, cells should be used promptly.
4. Incubate cell/DNA mix on ice for 30 minutes.
5. Heat shock cells by placing in a 42°C water bath for 30 seconds.
6. Return to ice for 2 minutes.
7. Add 250 µl room temperature SOC medium to each vial.
8. Secure the vials in a microcentrifuge tube rack with tape.
9. Place the rack, on its side, in a shaking incubator. Incubate at 37°C for 1 hour while shaking at 225–250 rpm.
10. Dilute the reaction containing the control pUC19 DNA 1:100 with SOC. Spread 50 µl of this dilution on LB plates containing 100 µg/ml ampicillin. NOTE: Spread the liquid gently and evenly over the surface of the agar using a glass spreader while turning the plate 3–5 rotations slowly on a turntable. Spreading the liquid until the surface of the plate is dry can significantly reduce the number of transformants.
11. Dilute the reactions containing experimental DNA as necessary and spread 100 to 200 µl on the appropriate selective plates.
12. Incubate at 37°C overnight.
13. Transformation efficiency (CFU/µg) may be calculated as follows:

$$\frac{\text{CFU on Plate}}{\text{pg pUC19 added}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{\text{tx vol}}{\text{vol plated}} \times \text{dilution factor} = \text{CFU}/\mu\text{g}$$

Example:

If a transformation experiment using 20 pg of pUC19 DNA results in 75 colonies after plating 50 µl of a 1:100 dilution, transformation efficiency is:

$$\frac{75 \text{ Colonies}}{20 \text{ pg}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{0.3 \text{ ml}}{0.05 \text{ ml}} \times 100 = 2.25 \times 10^9 \text{ CFU}/\mu\text{g}$$

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