canvax

DATA SHEET

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1. Identification

Product name **CVX5α CHEMICALLY COMPETENT CELLS**
40 rxn (4 vials x 500 μL)

Cat. No **C0031**

2. Description

Chemically competent cells are calcium chloridetreated to facilitate attachment of the plasmid DNA to the competent cell membrane. CVX5α Chemically Competent Cells provide a transformation efficiency of > 1 x 10^7 cfu/μg plasmid DNA. CVX5α is an economical solution for routine subcloning procedures or any application where the starting DNA is not limiting. CVX5α Competent Cells have similar properties as DH5α, which are suitable for high-efficiency transformation in a wide variety of applications. The φ80dlacZΔM15 marker provides α-complementation of the β-galactosidase gene from pUC or similar vectors and, therefore, can be used for blue/white screening of colonies on bacterial plates containing Bluo-gal or Xgal.

Genotype: F - , gyrA96, recA1, endA1, thi1, hsdR17(rK- mK +), deoR, supE44, Δ (lacZYA-argF)U169 ϕ80lacZΔM15 (similar to DH5α™).

3. Composition

Item	Quantity
Chemically Competent Cells	4 x 500 μL
pUC18 Transformation Control Plasmid	-

4. Quality Control

Each lot of competent cells is tested to verify transformation efficiencies using 10 ng pUC18 supercoiled DNA and the recommended protocol. Under these conditions, transformation efficiency will be ≥ $1x10^5$ cfu/μg pUC18.

Transformation efficiency is defined as the number of colonies forming units (cfu) which would be produced by transforming 1 μg of plasmid into a given volume of competent cells.

5. Handling and Storage specifications

- The CVX5α Chemically Competent Escherichia coli cells are shipped on dry ice.
- Upon receipt, store cells immediately at -80 ˚C. Storage at -20 ˚C will result in a significant decrease in transformation efficiency.
- Please note that competent cells are very sensitive to cycles of freezing and thawing and should not be exposed to temperature variations.

6. Further information

Product Use Limitations This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for any damage resulting from handling or from contact with the above product.

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Before you start

Use this procedure to transform CVX5α Competent Cells. We recommend verifying the transformation efficiency of the cells using the pUC18 control DNA supplied with the kit

STANDARD PROTOCOL FOR TRANSFORMATION

1. Prepare one LB/antibiotic/IPTG/X-Gal plate for each ligation reaction, plus one plate for determining transformation efficiency. Equilibrate the plates to room temperature prior to plating.

2. Centrifuge the tubes containing the ligation reactions or DNA to collect contents at the bottom of the tube. Add 10 µL of each ligation reaction or 10 ng of DNA sample to a sterile 1.5 mL microcentrifuge tube on ice. Set up another tube on ice **with 10 ng of pUC18 control** for determination of the transformation efficiency of the competent cells.

3. Remove a tube of frozen Competent Cells from storage at -80 ºC and place in an ice bath until just thawed (about 10 to 15 minutes). Mix the cells by **gently** flicking the tube with your fingertips.
IMPORTANT: Do not thaw competent cells with your hands. Keeping competent cells out of an ice bath even for extremely short times strongly affects the transformation efficiency of cells. Also avoid excessive pipetting, as the competent cells are extremely fragile and thus mixing of DNA with competent cells should be made by **gently** flicking and not by pipetting.

4. **Carefully** transfer 50 µL of cells into each tube prepared in **Step 2.** We recommend adding competent cells to ice-cooled microcentrifuge tubes containing DNA sample. If you prefer to add DNA sample to competent cells please make SURE your pipette tip goes all the way down in to the cells, so that you are adding DNA to the cells.

5. **Gently** flick the tubes to mix and place them on ice for 30 minutes.

6. Heat-shock the cells for exactly 45 seconds in a water bath at exactly 42 ˚C **(Do not shake nor heat shock more than 45 seconds).**

7. Immediately return the tubes to ice for 2 minutes.

8. Add 950 µl of room temperature SOC medium to the transformation reaction. Incubate for 1.5 hours at 37 ˚C with shaking (~250 rpm).

9. Plate 100 µl of each transformation reaction onto duplicate LB/ampicillin/IPTG/X-Gal plates.

10. Incubate plates overnight at 37 ˚C.

Calculation of transformation efficiency

Transformation efficiency (transformants/µg) is calculated as follows:

$$\frac{\textbf{CFU}}{\mu\textbf{g}} = \frac{x\ \textbf{CFU}}{\textbf{10 ng}} \times \frac{\textbf{1 x 10}^3\textbf{ng}}{\mu\textbf{g}} \times \frac{\textbf{1 mL}}{\textbf{0.1 mL}\ plated}$$