Corynebacterium glutamicum

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.510 – 12/2001

Microorganism	*Corynebacterium glutamicum*
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA
Growth medium	LBG (LB with 0.5% glucose) supplemented with 2.5% glycine and isonicotinic acid hydrazid (4 mg/ml)
Washing solution	15% glycerol (v/v)
Electroporation solution	15% glycerol (v/v)
Outgrowth medium	LBG medium (LB with 0.5% glucose)
Cuvette	2 mm gap width

Reference Haynes J. and Britz M. • 1990 • Journal of General Microbiology 136 • 255-263

Making electrocompetent cells:

1. Grow cells in LBG medium at 30 °C and shaking at 200 rpm to an $O.D._{600}$ of 0.15 to 0.25.
2. Harvest by centrifugation.
3. Wash in one culture volume of 15% glycerol.
4. Resuspend cells in 0.002 culture volumes of 15% glycerol (number of cells: approx. 2.5 x 10^{10} cells/ml).

Electroporation of cells:

1. Add 1-2 µl plasmid DNA (in water) to a minimum of 40 µl of electrocompetent cells. Homogenize by gently mixing with pipette several times. Transfer mixture into a prechilled cuvette.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:

Mode	Prokaryotes “O“
Voltage (V)	2,500 V
Time constant (τ)	5 ms

4. Immediately transfer cell suspension into LBG medium at a 1:25 dilution, incubate 1 hour at 30 °C.
5. Plate cells on selective plates; incubate for 4 days.

Expected results:

Transformation efficiency up to 5 x 10^5 transformants/µg of DNA.

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Eppendorf AG · 22331 Hamburg · Germany · Phone +49 40-5 38 01-0 · Fax +49 40-5 38 01-556

e-mail: eppendorf@eppendorf.com · Internet: www.eppendorf.com