Lactococcus lactis

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.519 – 12/2001

Microorganism	*Lactococcus lactis* MG1363
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA (pGK12)
Growth medium	Complex medium with 1% glycine
Washing solution	0.5 M sucrose, 10% glycerol
Electroporation solution	0.5 M sucrose, 10% glycerol
Outgrowth medium	Ice-cold complex medium with 0.5 M sucrose, 20 mM $MgCl_2$, 2 mM $CaCl_2$
Cuvette	1 mm gap width

Reference Dr. Horst Neve • Bundesanstalt für Milchforschung • Institut für Mikrobiologie
Hermann-Weigmann Str. 1 • D-24103 Kiel • Phone +49 431 6091 • Fax +49 431 609222

Making electrocompetent cells:

1. Grow cells overnight at 30 °C to an $O.D._{620}$ of 0.7.
2. Wash twice with ice-cold washing solution.
3. Resuspend cells in 1/100 volume of electroporation solution. Keep on ice.

Electroporation of cells:

1. Add 0.25 µg plasmid DNA (in water) to 100 µl of electrocompetent cells. Homogenize by gently mixing with pipette several times. Transfer mixture into cuvette.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:

Mode	Prokaryotes “O“
Voltage (V)	2,000 V
Time constant (τ)	5 ms

4. Add 1 ml ice-cold complex medium, incubate 2 hours at 30 °C.
5. Plate diluted cells on selective chloramphenicol plates. Incubate 2 days at 30 °C.

Expected results:

Transformation efficiency up to 1.4 x 10^6 transformants/µg of DNA.