Nocardia asteroides

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.525 – 01/2002

Microorganism	*Nocardia asteroides*
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA (in TE buffer)
Growth medium	N2 medium (20 g/l glucose, 2 g/l yeast extract, 4 g/l beef extract, 6 g/l tryptone, 2 g/l NaCl, 10 g/l glycine)
Washing solution	Distilled water
Electroporation solution	0.3 M sucrose, 15% glycerol
Outgrowth medium	N2 medium (without antibiotics)
Cuvette	2 mm gap width

Reference Yao W., et al • 1994 • Current Microbiology 29 • 223-227

Making electrocompetent cells:

1. Inoculate a fresh overnight culture of bacteria into N2 medium. Grow cells at 28 °C with shaking to an $O.D._{600}$ of 0.7.
2. Harvest by centrifugation.
3. Wash twice with ½ volume of distilled water.
4. Resuspend in a final volume of 1/20 of the original volume of electroporation solution.

Electroporation of cells:

1. Add 2 µl (0.1 µg) plasmid DNA to 50 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. Add 1 ml N2 medium and incubate for 2 hours at 30 °C.
5. Plate cells on selective YEME medium with a 3 ml YEME soft agar (0.4%) overlayment.

Expected results:

Transformation efficiency up to 8 x 10^4 transformants/µg of DNA.

eppendorf

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