Actinobacillus pleuropneumoniae

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.501 – 04/2002

Microorganism	*Actinobacillus pleuropneumoniae*
Cell type	Bacteria, gram negative
Molecules injected	Plasmid DNA (in water)
Growth medium	Columbia broth with 1% "IsoVitaleX and 10 µg/ml β-NAD
Washing solution	15% glycerine
Electroporation solution	15% glycerine
Outgrowth medium	Columbia broth with 1% "IsoVitaleX and 10 µg/ml β-NAD
Cuvette	2 mm gap width

Reference Frey, J. • 1992 • Research in Microbiology 143 • 263-269

Making electrocompetent cells:

1. Grow cells to mid-exponential growth phase at an $O.D._{650}$ of 0.5.
2. Harvest by centrifugation at 3,000 x g for 10 min at 4 °C.
3. Wash twice in 15% glycerine at 4 °C.
4. Resuspend in 1/20 volume of 15% glycerine and keep at 4 °C.

Electroporation of cells:

1. Add 3 µl (300 µg/ml) plasmid DNA to 125 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)	1,250 V
Time constant (τ)	5 ms

4. Immediately add 1 ml outgrowth medium and incubate for 3 h.
5. Plate onto selective Columbia agar plates.

Expected results:

Transformation efficiency up to 1 x 10^7 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