Listeria monocytogenes

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.520 – 12/2001

Microorganism	*Listeria monocytogenes* 23074
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA
Growth medium	BHI, supplemented with 0.5 M sucrose
Washing solution	1 mM HEPES (pH 7.0), 0.5 M sucrose
Electroporation solution	1 mM HEPES (pH 7.0), 0.5 M sucrose
Outgrowth medium	BHI, supplemented with 0.5 M sucrose
Cuvette	1 mm gap width

Reference Park, S. and Stewart, G. ♦ 1990 ♦ Gene 94 ♦ 129-132

Making electrocompetent cells:

1. Dilute an overnight culture of *L. monocytogenes* 23074 in BHI, into fresh media (1:100). Grow at 37 °C with shaking until reaching an $O.D._{600}$ of 0.2.
2. Add Penicillin G (10 µg/ml) and continue incubation for a further 2 hours.
3. Harvest by centrifugation (8,000 x g, 10 min., at 4 °C) and wash three times in ice-cold washing solution, once with equal volume and twice with ½ volume.
4. Resuspend the cells in ice-cold electroporation solution (0.0025 vol.). Use cells within 30 minutes of their preparation.

Electroporation of cells:

1. Add 1 µg plasmid DNA to 100 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)	1,000 V
Time constant (τ)	5 ms

4. Immediately add 1 ml of BHI medium and incubate at 37 °C for one hour.
5. Plate cells on selective BHI plates.

Expected results:

Transformation efficiency up to 4 x 10^6 transformants/µg of DNA.

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