Brucella abortus

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.508 – 12/2001

Microorganism	*Brucella abortus*
Cell type	Bacteria, gram negative
Molecules injected	Plasmid DNA (pBA∆*sod*kn^r)
Growth medium	Trypticase soy broth
Washing solution	Sterile, cold water
Electroporation solution	10% glycerol
Outgrowth medium	Trypticase soy broth
Cuvette	2 mm gap width

Reference Tatum F.M., et al ♦ 1992 ♦ Infection and Immunity 60 ♦ 2863-2869

Making electrocompetent cells:

1. Grow cells in Trypticase soy broth at 37 °C with vigorous shaking, chill on ice for 10 min.
2. Harvest by centrifugation (10 min, 10,000 x g). Wash three times in an equal volume of sterile cold water.
3. Resuspend cells in 1/500 volume of 10% glycerol (number of cells: 10^{10} cells/ml). Freeze aliquots on dry ice and store at –70 °C.

Electroporation of cells:

1. Add 10 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. Immediately add 1 ml trypticase soy broth, incubate 6 hours at 37 °C.
5. Plate cells on selective tryptose agar plates, incubate at 37 °C for four days.

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