Pseudomonas putida

Multiporator/Eppendorf Eporator®

Transformation Protocol **Protocol No. 4308 915.529 – 01/2002**

Microorganism	*Pseudomonas putida* P8
Cell type	Bacteria, gram negative
Molecules injected	Plasmid DNA
Growth medium	Standard 1-medium
Washing solution	10% glycerol
Electroporation solution	10% glycerol
Outgrowth medium	Standard 1-medium
Cuvette	2 mm gap width

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Making electrocompetent cells:

1. Inoculate 50 ml standard-1 medium with 7 ml of a fresh overnight culture of *Pseudomonas putida*. Grow cells at 30 °C to a density of O.D. of 0.8.
2. Harvest by centrifugation.
3. Wash twice with 50 ml ice-cold glycerol, centrifuge.
4. Resuspend cells in 0.8 ml ice-cold glycerol, keep on ice.

Electroporation of cells:

1. Add 4 µl plasmid DNA (1 µg) to 40 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)	2,400 V
Time constant (τ)	5 ms

4. Immediately add 1 ml standard 1-medium. Incubate 2 hours at 30 °C.
5. Plate cells on selective plates.

Expected results:

Transformation efficiency up to 2 x 10^4 transformants/µg of DNA.