Clostridium botulinum

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.509 – 12/2001

Microorganism	*Clostridium botulinum*
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA (pGK12 in water)
Growth medium	TPGY medium
Washing solution	10% PEG 8000
Electroporation solution	10% PEG 8000
Outgrowth medium	TPGY medium with 25 mM $MgCl_2$
Cuvette	4 mm gap width

Reference Zhou Y. and Johnson E. A. ♦ 1993 ♦ Biotechnology Letters ♦ 15, No. 2 ♦ 121-126

Making electrocompetent cells:

1. Grow cells in TPGY medium at 37 °C to an $O.D._{660}$ of 0.8.
2. Harvest by centrifugation. Wash in 0.8 volumes of PEG at 4 °C, keep cells cold during the entire procedure.
3. Resuspend cells in 0.8 ml 10% PEG (number of cells: 4 x 10^8 cells/ml), keep on ice.

Electroporation of cells:

1. Add 1 µg plasmid DNA to 800 µl of electrocompetent cells. Homogenize by gently mixing with pipette several times. Transfer mixture into a prechilled cuvette. Keep on ice for 2 min.
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. Dilute cells into 10 ml TPGY medium with 25 mM $MgCl_2$ and incubate 5 hours at 34 °C.
5. Plate cells on selective TPGY agar plates; incubate at 34 °C for 2-3 days.

Expected results:

Transformation efficiency up to 3 x 10^3 transformants/µg of DNA.

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