Gene Pulser® Electroprotocols

* We recommend adapting this protocol to use the Gene Pulser electroporation buffer (catalog #165-2676, 165-2677), which increases cell viability and transfection efficiency in mammalian cell lines.

Cell Type Mammalian, adherent, suspension

Species Used Mouse, NIH/3T3, embryo; Human T-cell line, (PEER)

Molecules Electroporated DNA: pTS1-envlF, 11 kB, supercoiled

Before the Pulse

Cell growth medium MDEM (GIBCO/BRL, Sigma)

Growth phase at harvest Not given

Pre-pulse incubation MDEM

Wash solution No

The Pulse

Electroporation Temperature 25 °C

Electroporation Medium* MDEM

Cell Density 75%

Volume of Cells 0.8 ml

DNA Concentration 1 μg / μl

DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH 8.0)

Volume of DNA 35 μl

Instruments Used Gene Pulser® apparatus & Capacitance Extender

Cuvette Gap 0.4 cm

Voltage 0.270 kV

Field Strength 0.675 kV/cm

Capacitor 960 μF

Resistor (Pulse Controller) Ω none

Time Constant 12.0 msec

After the Pulse

Outgrowth Medium MDEM

Outgrowth Temperature 25 °C

Length of Incubation 10 min.

Selection Method or Assay Used G418

Electroporation Efficiency Very good

Per Cent Survival 80 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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