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, suspension

Species Used Mouse, 32d, J558L, myeloma

Molecules Electroporated DNA: linear and circular plasmids, 4-12 kB in size

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Before the Pulse

Cell growth medium RPMI,10% Fetal Calf Serum GIBCO/BRL, Sigma)

Growth phase at harvest log

Pre-pulse incubation 10 minutes ice

Wash solution log

The Pulse

Electroporation Temperature (ice) 0 °C

Electroporation Medium* RPMI, 10% Nu-serum

Cell Density 10 (7) cells / μl

Volume of Cells 800 μl

DNA Concentration 1 mg / ml

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

Volume of DNA 20 to 40 μl

Instruments Used Gene Pulser® apparatus & Capacitance Extender, and Pulser Controller

Cuvette Gap 0.4 cm

Voltage 0.20 to 0.40 kV

Field Strength 0.5 to 1.0 kV/cm

Capacitor 960 μF

Resistor (Pulse Controller) none Ω

Time Constant 17 msec

After the Pulse

Outgrowth Medium Not given

Outgrowth Temperature Not given

Length of Incubation Not given

Selection Method or Assay Used G418; Hygromycin B

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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Date Submitted 3/5/91

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