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, J558-L, myeloma

Molecules Electroporated DNA: plasmids, 4.5 kB, supercoiled.

Before the Pulse

Cell growth medium DMEM + 10% Fetal Calf Serum (GIBCO/BRL, Sigma)

Wash solution None

Growth phase at harvest Split on the previous day

Pre-pulse incubation 10 min on ice

The Pulse

Electroporation Temperature 0 °C

Electroporation Medium* DMEM + 10% Fetal Calf Serum

Cell Density 10 (7) cells in 300 µl

Volume of Cells 300 µl

DNA Concentration 4 µg DNA per 10 (7) cells

DNA Resuspension Buffer Not given

Volume of DNA 4 µl

Instruments Used Gene Pulser® apparatus & Capacitance Extender

Cuvette Gap 0.4 cm

Voltage 0.25 kV

Field Strength 0.625 kV/cm

Capacitor 960 µF

Resistor (Pulse Controller) Ω none

Time Constant 50 msec

After the Pulse

Outgrowth Medium DMEM + 10% Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation 48 hours

Selection Method or Assay Used CAT assay

Electroporation Efficiency Not given

Per Cent Survival 60 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

PNAS, **87**: 5788 (1990).

Electroporation worked best for J558L cells - DEAE dextran did not; neither did lipofection.

Name of Submittor

Institution Address

Telephone Number

Fax Number

Date Submitted 8/23/90

Survey Number 135