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

Species Used Rat, CA77, medullary thyroid carcinoma cell line

Molecules Electroporated DNA: supercoiled plasmid; luciferease reporter gene

Before the Pulse

Cell growth medium DMEM/F12 (1:1) + 10 % Fetal Bovine Serum (GIBCO/BRL, Sigma)

Wash solution Dulbecco's Phosphate Buffered Saline (minus Ca^{++}, Mg^{++})

Growth phase at harvest Active growth, about 50 to 70 % confluent

Pre-pulse incubation None

The Pulse

Electroporation Temperature Room temperature

Electroporation Medium* Dulbecco's Phosphate Buffered Saline (minus Ca^{++}, Mg^{++})

Cell Density 4 to 5 x10 (6) cells / 800 μl

Volume of Cells 800 μl

DNA Concentration 5 to 20 μg

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

Volume of DNA 5 to 50 μl (usually less than 10 μl)

Instruments Used Gene Pulser® apparatus & Capacitance Extender

Cuvette Gap 0.4 cm

Voltage 0.22 kV

Field Strength 0.55 kV/cm

Capacitor 960 μF

Resistor (Pulse Controller) Ω none

Time Constant about 12 msec

After the Pulse

Outgrowth Medium DMEM/F12 (1:1) + 10 % Fetal Bovine Serum

Outgrowth Temperature 37 °C

Length of Incubation usually 24 hours

Selection Method or Assay Used Luciferase assay, β-galactosidase assay

Electroporation Efficiency about 1 x 10 (4) / μg [20 to 40 % cells stained blue, β-gal, transient assay]

Per Cent Survival 50 to 80 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Reference: Tverberg, L.A. and Russo, A.F. 1992. *J. Biol. Chem.* **267**(5):17567-17573.

Name of Submittor

Institution Address

Telephone Number

Fax Number

Date Submitted 9/4/92

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