



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 Human, HeLa, epithelial carcinoma

Molecules Electroporated DNA: pBLCAT2 series, 4.5 kB

Before the Pulse

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

Growth phase at harvest Log phase

Pre-pulse incubation 5 minutes on ice

Wash solution Phosphate Buffered Saline

The Pulse

Electroporation Temperature 4 °C (from ice to chamber at 25 °C)
Electroporation Medium* Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus & Capacitance Extender

Cell Density 2 x 10⁽⁶⁾ cells in cuvette

Cuvette Gap 0.4 cm

Volume of Cells 0.8 ml

Voltage 0.250 kV

DNA Concentration 2 to 20 µg

Field Strength 0.625 kV/cm

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

Capacitor 960 µF

Volume of DNA 2 to 20 µl

Resistor (Pulse Controller) Ω none

Time Constant 18 to 20 msec

After the Pulse

Outgrowth Medium MEM + 10% Fetal Calf Serum

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C

Nucleic Acid Research, **18**(3): 465-470 (1990).

Length of Incubation 40 hours

PBS: 1x = 8g NaCl, 0.2g KCl, 0.2g KH₂PO₄, 1.15g Na₂HPO₄

Selection Method or Assay Used CAT assay

Electroporation Efficiency Not determined

Per Cent Survival 20 to 60 %

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 10/23/90

Survey Number 096

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