



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 Ovine (sheep), R.E., rumen

Molecules Electroporated DNA: supercoiled DNA used for transient transfections.

Before the Pulse

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

Growth phase at harvest 50 to 70% confluency

Wash solution Wash two times in electroporation buffer.

Pre-pulse incubation 4° C, 10 min. (optional: add 50 µl FCS if using HeBS as electroporation media; 50 µl salmon sperm DNA for transient transfections).

The Pulse

Electroporation Temperature Room temperature

Instruments Used Gene Pulser® apparatus & Capacitance Extender

Electroporation Medium* HEPES Buffered Saline, 6mM glucose, (optional: add 50 µl FCS, 50 µl salmon sperm DNA).

Cell Density 5 x 10⁶ cells / pulse

Cuvette Gap 0.4 cm

Volume of Cells 0.5 ml

Voltage 0.270 kV

DNA Concentration 10 µg / pulse

Field Strength 0.675 kV/cm

DNA Resuspension Buffer Not given; pulse volume: 0.8 ml

Capacitor 960 µF

Volume of DNA Not given; pulse volume: 0.8 ml

Resistor (Pulse Controller) Ω none

Time Constant Not given

After the Pulse

Outgrowth Medium DMEM, 10% Fetal Calf Serum (FCS)

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C

HBS: 10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM CaCl₂

Length of Incubation 48 to 72 hrs.

Selection Method or Assay Used Transient assays

Electroporation Efficiency Not given

Per Cent Survival about 50 %

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 7/1/90

Survey Number 151

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