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 Human, MRC-5 / V1, lung fibroblasts, transformed

Molecules Electroporated DNA: neo gene.

Before the Pulse

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

Growth phase at harvest Log phase

Pre-pulse incubation 37° C, 1/2 hour

Wash solution Phosphate Buffered Saline

The Pulse

Electroporation Temperature 25°C

Electroporation Medium* DMEM

Cell Density 5 x 10 (6) cells

Volume of Cells 1.4 ml (**see notes)

DNA Concentration 20 μg

DNA Resuspension Buffer water

Volume of DNA 20 μl

Instruments Used Gene Pulser® apparatus

Cuvette Gap 0.4 cm

Voltage 2.0 kV

Field Strength 5.0 kV/cm

Capacitor 25 μF

Resistor (Pulse Controller) Ω none; NOT RECOMMENED***(see notes)

Time Constant 0.4 msec

After the Pulse

Outgrowth Medium DMEM, 10% Fetal Calf Serum

Outgrowth Temperature 37 ° C

Length of Incubation 1 month for selection

Selection Method or Assay Used 1 mg / ml geneticin

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller. It is not recommended to use more than 0.8 ml in the 0.4 cm cuvette; greater volumes may create non-uniform field strengths during the pulse.

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