

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

Hybrid, mouse/human, A9 fibroblast,

Species Used hybrid containing human chromosomes

Molecules DNA: linearized 8.5 kB plasmid

Electroporated

Before the Pulse

Cell growth medium DMEM + 10% Fetal Calf Serum +

mycophenolic acid + xanthine

Growth phase at harvest 10 (7) cells / ml

Used Capacitance Extender

0. 250, 0.450 kV

0.625,1.125 kV/cm

125, 250, 500, 960 μ F

(Pulse Controller) Ω none

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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

0.4 cm

Pre-pulse incubation

Instruments

Cuvette Gap

Voltage

Strength

Capacitor

Resistor

Time

Field

10 min. on ice, (0°C)

Gene Pulser® apparatus &

Wash solution **HEPES Buffered Saline (HBS)**

The Pulse

Electroporation Room temperature

Temperature

HEPES Buffered Saline (HBS)

Medium*

Electroporation

Approximately 10 (7) cells / ml **Cell Density**

 $0.8 \, \mathrm{ml}$ Volume of Cells

 $10 \mu g$ **DNA** Concentration

DNA Resuspension

Buffer

Not given

Not given Volume of DNA

Not given Constant

After the Pulse

Not given **Outgrowth Medium**

37°C **Outgrowth Temperature**

Not given Length of Incubation

Selection Method or **Assay Used**

Hygromycin B

Electroporation

Efficiency

Not given

Per Cent Survival

Not given

Name of **Submittor** Institution Address

Telephone Number

Fax Number

5 mM CaCl2

Date Submitted 8/20/90

Survey Number 165

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