



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 Hamster, CHO, ovary

Molecules Electroporated DNA: supercoiled DNA used for transient transfections; linearized DNA used for stable transfections.

Before the Pulse

Cell growth medium F12, 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. (option: add 50µl FCS if using HeBS as electroporation media; 50 µl salmon sperm DNA for transient transfections).

The Pulse

Electroporation Temperature Room temperature
Electroporation Medium* Phosphate Buffered Saline

Instruments Used Gene Pulser® apparatus & Capacitance Extender

Cell Density 5 x 10 (6) cells/pulse for transient assay; 5 x 10 (5) cells/pulse, stable transfection

Cuvette Gap 0.4 cm

Volume of Cells 0.5 ml

Voltage 1.30 kV

DNA Concentration 10 µg / pulse

Field Strength 3.25 kV/cm

DNA Resuspension Buffer Not given; final volume: 0.8 ml

Capacitor 25 µF

Volume of DNA Not given; final volume: 0.8 ml

Resistor (Pulse Controller) Ω none. NOT RECOMMENDED*** (see notes)

Time Constant 0.4 msec

After the Pulse

Outgrowth Medium F12, 10% Fetal Calf Serum (FCS)

Relevant Publications and/or Comments

Outgrowth Temperature 37 °C

Note: exponential values designated in parentheses.
**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

Length of Incubation 48 to 72 hrs.

Note: Stable transfections generally do not use carrier DNA. Also, the level of selective agent required to kill off non-transfected cells needs to be established before transfection. The level required should kill non-transfected cells in approximately 7 days.

Selection Method or Assay Used G418 (stable transfections) and transient assays

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

Electroporation Efficiency Not given

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

Per Cent Survival about 50%

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 7/1/90

Survey Number 087

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