



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 Proteins: restriction enzymes (*Eco* RI, *Sca*I, *Dra*I) and catalase

Before the Pulse

Cell growth medium McCoy's 5a (GIBCO/BRL, Sigma) **Growth phase at harvest** Exponential
Wash solution Phosphate Buffered Saline (PBS) or serum-free medium **Pre-pulse incubation** Hepes Buffered Saline (HBS)

The Pulse

Electroporation Temperature 4°C **Instruments Used** Gene Pulser® apparatus & Capacitance Extender
Electroporation Medium* HEPES Buffered Saline (HBS)
Cell Density 2 x 10⁽⁶⁾ cells / ml **Cuvette Gap** 0.4 cm
Volume of Cells 0.8 ml **Voltage** 0.3 kV
DNA Concentration Not given **Field Strength** 0.750 kV/cm
DNA Resuspension Buffer Not given **Capacitor** 960 µF
Volume of DNA Not given **Resistor** (Pulse Controller) Ω none
Time Constant 16 to 22 msec

After the Pulse

Outgrowth Medium McCoy's 5A **Relevant Publications and/or Comments**
Outgrowth Temperature 37 °C **Note:** exponential values designated in parentheses. Cortez, F., and Ortiz, T. *Mutation Research* 246(1):221-6
Length of Incubation 20 hr. **PBS:** 1x = 8g NaCl, 0.2g KCl, 0.2g KH₂PO₄, 1.15g Na₂HPO₄
Selection Method or Assay Used Not given **HBS:** 10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM CaCl₂
Electroporation Efficiency Not given
Per Cent Survival 40 to 60 %

Name of Submitter
Institution Address

Telephone Number
Fax Number
Date Submitted 11/15/90
Survey Number 090