



# 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 KH<sub>2</sub>PO<sub>4</sub>, 1.15g Na<sub>2</sub>HPO<sub>4</sub>

**Electroporation Efficiency** Not given

**HBS:** 10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM CaCl<sub>2</sub>

**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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