



# 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, suspension  
**Species Used** Mouse, D10.G4.1, T-cell, helper

**Molecules Electroporated** DNA

## Before the Pulse

**Cell growth medium** DMEM (10% fetal calf serum) + 20% con A supplement (GIBCO/BRL, Sigma)

**Growth phase at harvest** log phase

**Pre-pulse incubation** 10 to 15 min on ice

**Wash solution** Phosphate Buffered Saline without Ca<sup>++</sup>, Mg<sup>++</sup>

## The Pulse

**Electroporation Temperature** Room Temperature  
**Electroporation Medium\*** Ca<sup>++</sup>, Mg<sup>++</sup> free

**Instruments Used** Gene Pulser® apparatus & Capacitance Extender

**Cell Density** 10 (7) cells / 800 µl

**Cuvette Gap** 0.4 cm

**Volume of Cells** 800 µl

**Voltage** 0.3 kV

**DNA Concentration** 10 µg in sterile water or TE

**Field Strength** 0.95 kV/cm

**DNA Resuspension Buffer** Not given

**Capacitor** 960 µF

**Volume of DNA** 10 to 20 µl

**Resistor** (Pulse Controller) Ω none

**Time Constant** 11 msec

## After the Pulse

**Outgrowth Medium** DMEM (10% serum) + 20% con A supplement

### Relevant Publications and/or Comments

**Outgrowth Temperature** 37 °C

**Note:** exponential values designated in parentheses.

**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>

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

**Length of Incubation** 2 days

**Selection Method or Assay Used** Hygromycin

**Electroporation Efficiency** not quantified (used CAT assay)

**Per Cent Survival** 70 to 80 %

**Name of Submitter**  
**Institution Address**

**Telephone Number**

**Fax Number**

**Date Submitted** 8/27/90

**Survey Number** 143

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