# 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 | **Molecules Electroporated** | Proteins |
| **Species Used** | Monkey, CV-1, kidney; Mouse, 3T3, embryo; Mouse, p3x63AG8, myeloma; Human: HeLa, epithelial carcinoma | | |

## Before the Pulse

| | | | |
|---|---|---|---|
| **Cell growth medium** | DMEM (GIBCO/BRL, Sigma) | **Growth phase at harvest** | 50 to 60% confluent cells |
| | | **Pre-pulse incubation** | 5 minutes |
| **Wash solution** | (See notes) | | |

## The Pulse

| | | | |
|---|---|---|---|
| **Electroporation Temperature** | 0 °C | **Instruments Used** | Gene Pulser® apparatus |
| **Electroporation Medium*** | (See notes) | | |
| | | **Cuvette Gap** | 0.4 cm |
| **Cell Density** | 5 x 10 (6) cells / ml | **Voltage** | 0.5 to 0.75 kV |
| **Volume of Cells** | 0.2 $\mu$l | **Field Strength** | 1.25 to 1.875 kV/cm |
| **DNA Concentration** | Not given | **Capacitor** | 25 $\mu$F |
| **DNA Resuspension Buffer** | Not given | **Resistor** | (Pulse Controller) Ω none |
| **Volume of DNA** | Not given | **Time Constant** | 2 to 4 msec |

## After the Pulse

| | |
|---|---|
| **Outgrowth Medium** | Pore-Resealing Buffer (see notes) for 10 min. ,37° C; follow with DMEM plus 10% Fetal Calf Serum. |
| **Outgrowth Temperature** | 37° C |
| **Length of Incubation** | Not given |
| **Selection Method or Assay Used** | Not given |
| **Electroporation Efficiency** | Not given |
| **Per Cent Survival** | Not given |

**Relevant Publications and/or Comments**

**Note:** exponential values designated in parentheses.
**Ref**:M.R. Mitchell et. al. (1988) *Experentia* **44**: 199-203.
M.R. Michel *et. al.* (1990) *J.Virol.* **64**: 5123-5131.
Elgizoli, M. *et. al.* (1989) *J. Virol.* **63**: 2921-2928.
**Electroporation Buffer:** 20 mM PIPES, pH 7; 128 mM K-glutamate, 5mM ATP, 5 mM GTP, 10 $\mu$m Ca-Acetate, 2 mM Mg-Acetate & amino acid concentration corresponding to that of DMEM media.
**Pore-Resealing Buffer:** 20 mM PIPES, pH 7.0, 128 mM K-glutamate,10 $\mu$m Ca-Acetate, 2 mM Mg-Acetate

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