



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 | Molecules Electroporated | DNA: multiple CAT and other vectors under different promoters including HIV, SIV, human and rhesus cytomegalovirus immediate early gene, SV40 and the simian retrovirus type 2. |
| Species Used | Monkey, COS-7, kidney, SV-40 transformed | | |

Before the Pulse

| | | | |
|---------------------------|---|--------------------------------|--|
| Cell growth medium | RPMI 1640 with 10 mM dextrose and 0.1mM dithiothreitol (Sigma, GIBCO/ BRL, Flow Labs) | Growth phase at harvest | Log phase (We routinely subdivide cells 24 hours prior to electroporation) |
| Wash solution | Trypsin harvest, washed twice in Phosphate Buffered Saline | Pre-pulse incubation | None |

The Pulse

| | | | |
|------------------------------------|---|-------------------------|---|
| Electroporation Temperature | 25 °C | Instruments Used | Gene Pulser® apparatus & Capacitance Extender |
| Electroporation Medium* | RPMI 1640 without Fetal Calf Serum, +10mM dextrose, 0.1 mM dithiothreitol | Cuvette Gap | 0.4 cm |
| Cell Density | 1.3 x 10 ⁽⁷⁾ viable cells / ml, 0.3 ml | Voltage | 0.30 kV |
| Volume of Cells | 300 µl | Field Strength | 0.75 kV/cm |
| DNA Concentration | 500 µg / ml (5 to 10 µg per pulse) | Capacitor | 960 µF |
| DNA Resuspension Buffer | Not given | Resistor | (Pulse Controller) Ω none |
| Volume of DNA | 10 to 20 µl | Time Constant | 33 to 38 msec |

After the Pulse

| | |
|---------------------------------------|--|
| Outgrowth Medium | DMEM culture media, 10% fetal calf serum |
| Outgrowth Temperature | 37 °C |
| Length of Incubation | 48 hours |
| Selection Method or Assay Used | Transient (CAT, β-gal, immunohisto-chemistry). |
| Electroporation Efficiency | 50 to 100% |
| Per Cent Survival | 20 to 75 % |

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. All plasmids were prepared by alkaline lysis and banded to equilibrium twice in cesium chloride gradients. Largest DNA molecule electroporated has been the rhesus cytomegalovirus genome (approximately 225 kilobases). In general, the higher the efficiency of transfection, the higher degree of cell killing. For some cell types, > 90% of the input cells are killed, although > 90% of the survivors express very high levels of input DNA. We have found that ≥ 20 µg of DNA per electroporation resulted in greatly reduced efficiency of transfection. Similarly, 2.5 µg of DNA resulted in reduced efficiency.

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