



# 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.

|                     |                                   |                                 |   |
|---------------------|-----------------------------------|---------------------------------|---|
| <b>Cell Type</b>    | Mammalian, suspension             | <b>Molecules Electroporated</b> | DNA: plasmid, closed circular, CsCl purified x2; Co-transfection with 2 plasmids; pHIV LTR-CAT and pSV40-Tat; also transfection with pCH110 alone (7.2 kB). |
| <b>Species Used</b> | Human, U937, hystiocytic lymphoma |                                 |   |

## Before the Pulse

|                           |   |                                |                 |
|---------------------------|---|--------------------------------|-----------------|
| <b>Cell growth medium</b> | RPMI 1640 + 10% fetal calf serum + penicillin/streptomycin, L-glutamine, sodium pyruvate (GIBCO/BRL, Sigma) | <b>Growth phase at harvest</b> | Log growth      |
| <b>Wash solution</b>      | Cell growth media   | <b>Pre-pulse incubation</b>    | 10 min at 4° C. |

## The Pulse

|                                    |                              |                         |   |
|------------------------------------|------------------------------|-------------------------|---|
| <b>Electroporation Temperature</b> | 4°C                          | <b>Instruments Used</b> | Gene Pulser® apparatus & Capacitance Extender |
| <b>Electroporation Medium*</b>     | Cell growth media            | <b>Cuvette Gap</b>      | 0.4 cm  |
| <b>Cell Density</b>                | 1.3 x 10 <sup>(7)</sup> / ml | <b>Voltage</b>          | 0.30 kV                                       |
| <b>Volume of Cells</b>             | 250 µl                       | <b>Field Strength</b>   | 0.75 kV/cm                                    |
| <b>DNA Concentration</b>           | Not given                    | <b>Capacitor</b>        | 960 µF  |
| <b>DNA Resuspension Buffer</b>     | Not given                    | <b>Resistor</b>         | (Pulse Controller) Ω none                     |
| <b>Volume of DNA</b>               | Not given                    | <b>Time Constant</b>    | Not given                                     |

## After the Pulse

|                                       |                                      |  |  |
|---------------------------------------|--------------------------------------|--|--|
| <b>Outgrowth Medium</b>               | Cell growth media                    | <b>Relevant Publications and/or Comments</b> | <b>Note:</b> exponential values designated in parentheses. |
| <b>Outgrowth Temperature</b>          | 37°C                                 |  |  |
| <b>Length of Incubation</b>           | 24 to 48 hr.                         |  |  |
| <b>Selection Method or Assay Used</b> | CAT assay, stain for β-galactosidase |  |  |
| <b>Electroporation Efficiency</b>     | About 500 transfectants / µg DNA     |  |  |
| <b>Per Cent Survival</b>              | 15 %                                 |  |  |

|                            |                               |
|----------------------------|-------------------------------|
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