



# 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</b>      | DNA: pRSVneo, linearized, 5.6 kB |
| <b>Species</b>   | Human, K562, chronic myeloid | <b>Electroporated</b> |                                  |
| <b>Used</b>      | leukemia                     |                       |                                  |

## Before the Pulse

|                           |  |                                |           |
|---------------------------|--|--------------------------------|-----------|
| <b>Cell growth medium</b> | RPMI + 20% Fetal Calf Serum (GIBCO/BRL, Sigma) | <b>Growth phase at harvest</b> | Log       |
| <b>Wash solution</b>      | RPMI   | <b>Pre-pulse incubation</b>    | Not given |

## The Pulse

|                                    |                                 |                         |   |
|------------------------------------|---------------------------------|-------------------------|---|
| <b>Electroporation Temperature</b> | Room temperature                | <b>Instruments Used</b> | Gene Pulser® apparatus & Capacitance Extender |
| <b>Electroporation Medium*</b>     | RPMI                            | <b>Cuvette Gap</b>      | 0.4cm   |
| <b>Cell Density</b>                | 5 x10 <sup>(6)</sup> cells / ml | <b>Voltage</b>          | up to 2 kV                                    |
| <b>Volume of Cells</b>             | 0.4 to 0.8 ml                   | <b>Field Strength</b>   | up to 5 kV/cm                                 |
| <b>DNA Concentration</b>           | 5 µg / 800 µl                   | <b>Capacitor</b>        | up to 960 µF                                  |
| <b>DNA Resuspension Buffer</b>     | Not given                       | <b>Resistor</b>         | (Pulse Controller) Ω none                     |
| <b>Volume of DNA</b>               | up to 50 µl                     | <b>Time Constant</b>    | 0.4 msec                                      |

## After the Pulse

|                                       |                             |  |  |
|---------------------------------------|-----------------------------|--|--|
| <b>Outgrowth Medium</b>               | RPMI + 20% Fetal Calf Serum | <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>           | 48 hr.                      |  |  |
| <b>Selection Method or Assay Used</b> | G418                        |  |  |
| <b>Electroporation Efficiency</b>     | 0.3% clonogenic cells / µg  |  |  |
| <b>Per Cent Survival</b>              | Not given                   |  |  |

|                            |                                |
|----------------------------|--------------------------------|
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|                            | <b>Date Submitted</b> 10/15/90 |
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