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, FDC-PI, Il-3-dependent cell line

Molecules Electroporated DNA: 6.5 kB, linearized plamid.

Before the Pulse

Cell growth medium RPMI 1640, 10% Fetal Calf Serum (GIBCO/BRL, Sigma)

Wash solution HEPES buffered saline (see notes)

Growth phase at harvest Not given

Pre-pulse incubation Ice, 10 min.

The Pulse

Electroporation Temperature 0 °C

Electroporation Medium* HEPES buffered saline

Cell Density 1 x 10 (7) cells / ml

Volume of Cells 0.5 ml

DNA Concentration 1 mg / ml

DNA Resuspension Buffer TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0)

Volume of DNA 10 μl (10 μg) / pulse

Instruments Used Gene Pulser® apparatus

Cuvette Gap 0.4 cm

Voltage 1.5 kV

Field Strength 3.75 kV/cm

Capacitor 25 μF

Resistor (Pulse Controller) Ω none. NOT RECOMMENED***(see notes)

Time Constant 0.8 msec

After the Pulse

Outgrowth Medium RPMI 1640, 10 % Fetal Calf Serum

Outgrowth Temperature 37 °C

Length of Incubation Not given

Selection Method or Assay Used G418, 400 μg / ml

Electroporation Efficiency 10 to 20 transfectants / μg DNA

Per Cent Survival 60% at 10 min. after electroporation

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Ref: *EMBO J.* **9** : 4367-4374 (1990) Molecular cloning and expression of the murine interlenkin -5 receptor.

HEPES Buffered Saline: 140 mM NaCl, 5 mM KCl, 0.75 mM Na2HPO4, 6 mM dextrose, 25 mM HEPES, pH 7.2

**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

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