



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	DNA: linearized plasmid DNA; RSV LTR promoting human HLA Class II genes, along with HTK promoter (neo r gene) containing plasmid (co-transfection)
Species Used	Human, K562, chronic myeloid leukemia; U937, histiocytic lymphoma		

Before the Pulse

Cell growth medium	RPMI 1640 + 10% Fetal Calf Serum (GIBCO/ BRL, Sigma)	Growth phase at harvest	Early log phase growth
Wash solution	RPMI without serum	Pre-pulse incubation	10 min. on ice

The Pulse

Electroporation Temperature	4 °C	Instruments Used	Gene Pulser® apparatus & Capacitance Extender
Electroporation Medium*	RPMI without serum	Cuvette Gap	0.4 cm
Cell Density	10 (7) cells / ml	Voltage	0.2 kV
Volume of Cells	0.4 ml	Field Strength	0.5 kV/cm
DNA Concentration	1 µg / µl	Capacitor	960 µF
DNA Resuspension Buffer	TE (10 mM Tris, 1 mM EDTA, pH 8.0)	Resistor	(Pulse Controller) none Ω
Volume of DNA	20 µl	Time Constant	15 to 20 msec

After the Pulse

Outgrowth Medium	RPMI 1640 + 10% Fetal Calf Serum
Outgrowth Temperature	37 °C
Length of Incubation	15 to 30 days
Selection Method or Assay Used	G418 resistance
Electroporation Efficiency	Unknown (see notes)
Per Cent Survival	<1 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. Cells resistant to G418 (1µg/ml) grew out of post-pulse cell culture after about 15-30 days, with low % of survival. G418 resistant cells were then dilution-cloned in preparation for screening a Class II expressing clone. However, before the majority of cells could be screened, the clones were lost. I don't know how efficient electroporation procedure was since we were trying to get expression of a two-chain molecule on the cell surface - many factors could go wrong post-electroporation but prior to protein expression on the cell surface.

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