

# Optimization Protocol – Cell Lines

Multiporator®

Transfection Protocol

09/2003

Parameters for the electroporation of eukaryotic cell lines can be found in our existing protocols. These values may also be used if it is certain that a special cell type behaves similarly to the cell type from the protocol. When using special cell lines, we recommend the optimization of single parameters according to the following guideline. A standard procedure for an electroporation experiment is provided by the General Protocol for cell lines (available at [www.eppendorf.com](http://www.eppendorf.com)).

## 1. Temperature

Experiments to establish new protocols normally take place at room temperature. As an alternative method for further optimization, electroporation may be carried out at 4 °C (see point 6).

## 2. Adjustment of the electroporation buffer

Ideally, electroporation should be carried out in hypoosmolar buffer. Testing the tolerance of the cells is done by incubation for 30 min in the hypoosmolar buffer and then performing a viability stain. If the survival rate of the cells is > 90 %, the hypoosmolar buffer can be used in undiluted form for electroporation.

For sensitive cells undergoing lysis, the osmolarity should be increased by gradually mixing the hypoosmolar buffer with the isoosmolar buffer until the survival rate of the cells exceeds 90 %.

It is essential to ensure that the cells do not remain in the electroporation buffer for longer than 30 minutes.

## 3. Electroporation parameter

The variable electrical parameters are field strength [kV/cm], pulse length [µsec] and pulse number. The field strength is dependent upon the voltage set on the device and the distance on the electrodes (gap width of cuvette).

### 3.1 Calculating the critical field strength and the voltage to be set on the device

As the size of the cell is a crucial factor for setting parameters on the Multiporator, it should be estimated after an incubation of 10-15 min in the electroporation buffer.

Based on the diameter, the critical field strength can be approximately calculated using the following formula:

$$E_c = V_c / (0.75 \times d_{\text{cell}})$$

$E_c$ : Critical field strength [V/cm]

$V_c$ : Permeation voltage of the membrane [1 V at 20 °C; 2 V at 4 °C]

$d_{\text{cell}}$ : Cell diameter [cm]

For calculating the voltage to be set on the Multiporator, the field strength  $E_c$  is multiplied by the gap width of the cuvette.

$$V = E_c \times d_{\text{cuv}}$$

$V$ : Voltage (setting on Multiporator) [V]

$E_c$ : Critical field strength [V/cm]

$d_{\text{cuv}}$ : Electrode distance (cuvette type) [cm]

Cell diameter	Voltage 2 mm cuvette RT	Voltage 4 mm cuvette RT	Voltage 2 mm cuvette 4 °C	Voltage 4 mm cuvette 4 °C
5 µm	530	1070	1070	*
10 µm	270	530	530	1070
15 µm	180	360	360	710
20 µm	130	270	270	530
25 µm	110	210	210	430
30 µm	90	180	180	360

\* The maximum voltage attainable with the eukaryotic module of the Multiporator is 1,200 V.

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## 3.2 Testing of different field strengths

The critical field strength is the minimum value at which the membrane can be permeated. To determine the optimal field strength, it is advisable to carry out a series of experiments in which the minimum value, twice the value and then three times the value are used for suspension cells, and up to five times the value for adherent cells.

## 3.3 Testing of different pulse lengths

Empirically, the ideal pulse lengths for electroporation have proven to be 40-100 µsec at RT and 15-40 µsec at 4 °C. To optimize the time constants, three different pulse lengths should be chosen within the above-mentioned ranges.

## 3.4 Variation of the number of field pulses

For most cell lines, electroporation is carried out with one pulse. If one pulse proves to be insufficient, two or more pulses may be used. During multiple pulsing, the Multiporator automatically maintains a 60-second interval between pulses to allow the cell membrane to regenerate.

## 4. Determination of the transfection efficiency

Depending on the cell type and on the plasmid used, transient expression may be roughly detected 24-48 hours after transfection. In some cases (e.g. primary cells), this may take considerably longer.

## 5. Analyzing the result and optimization

The aim of an optimization procedure is to achieve a compromise between effective transfection and high survival rate of the cells.

After performing the first series of experiments, parameters can often be found with which it is possible to obtain a high transfection rate. If required, further tests can be carried out with a selection of field strengths surrounding the best result from the first series. The pulse length and the number of pulses can also be varied at this point.

If the survival rate of the cells should be increased, parameters can be changed by trying lower field strengths, shorter pulses and a reduced number of pulses. Even a shorter incubation time of the cells in electroporation buffer should be considered.

Optimizing for greater transfection efficiency is carried out by choosing higher field strengths, longer pulses and multiple pulsing. Higher DNA concentrations or lower cell numbers can also be used, and the incubation period for gene expression may be lengthened.

## 6. Higher efficiency at lower temperatures

With certain cell types, electroporation at low temperatures (e.g. 4 °C) can lead to an increase in the amount of transfection material absorbed. The healing process of the cell membrane is slowed down at lower temperatures.

In cases where electroporation is performed at 4 °C, the survival rate of the cells can be increased by the following procedure: resuspended cells in electroporation buffer at 37 °C or room temperature, cool down to 4 °C and then transfer into precooled cuvettes. Following electroporation, incubate cells at 4 °C for a maximum of two minutes and then heat to 37 °C.

**Further reading:** Basic Application Manual and General Protocol (available on [www.ependorf.com](http://www.ependorf.com))

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