Multiporator optimization

Buffer preparation

Before you start, you need to prepare the electroporation buffer:

	Poration (hypoosmolar)	Poration (isoosmolar)
Sterile bidistilled water	Fill up to 1000 ml	Fill up to 1000 ml
KCl	25 mM	25 mM
KH_2PO_4	0.3 mM	0.3 mM
K_2HPO_4	0.85 mM	0.85 mM
myo-Inositol *	ad 90 mOsmol/kg	ad 280 mOsmol/kg
pH value	7.2 ± 0.1	7.2 ± 0.1
Conductivity at 25 °C	3.5 mS/cm ± 10 %	3.5 mS/cm ± 10 %

*The purity of myo-Inositol may vary greatly from batch to batch. It must be pure enough to ensure that, at 280 mOsmol/kg in bidistilled water, a conductivity of 10 µS/cm is not exceeded. The conductivity of individual myo-Inositol batches should be measured before the buffer is prepared.

Testing the tolerance of the cells to hypoosmolar conditions:

incubating the cells for 30 minutes in hypoosmolar buffer.

performing a viability stain using trypan blue or propidium iodide. If observation under a microscope reveals lysis in more than 10 % of the cells, the osmolarity of the buffer must be increased by adding isoosmolar buffer. To determine the optimal osmolarity, it is advisable to incubate the cells in different mixing ratios of hypo- and isoosmolar buffer for 30 minutes prior to the experiment (see table below). This 30-minute period is the maximum incubation time for the cells in the electroporation buffer system. A new viability test followed by observation under a microscope determines the osmolarity that can be tolerated by the cells. The mixing concentrations can then be used for all subsequent experiments with this cell type.

Volumes of Eppendorf Hypoosmolar and Isoosmolar Electroporation Buffers to be used to adjust the desired osmolarity (final volume: 10 ml):

Desired osmolarity	Eppendorf Hypoosmolar Buffer (ml)	Eppendorf Isoosmolar Buffer (ml)
90 mOsmol/kg	10	0
150 mOsmol/kg	6.8	3.2
200 mOsmol/kg	4.2	5.8
250 mOsmol/kg	1.6	8.4
280 mOsmol/kg	0	10

Determining the diameter of the cell:

Incubate cells in electroporation buffer for 10-15 minute.

Measure the diameter of the cell using measuring eyepiece.

Electroporation procedure:

1. Ensure that cells are harvested in the exponential growth phase.
2. Dilute the cells in culture medium with 0.5 to 1 % FCS and determine the number of cells and spin the cells down.
3. Resuspend the cells in Eppendorf Electroporation Buffer (at RT or 4 °C) with the determined osmolarity and set a cell concentration of between 1 x 10^6 and 3 x 10^6 cells/ml, or slightly lower.

Caution: The overall incubation time in the Eppendorf Electroporation Buffer must not exceed 30 minutes to guarantee successful electroporation!

4. Aliquot the cell suspension (400 µl for a cuvette with 2-mm gap width and 800 µl for a cuvette with 4-mm gap width) in Eppendorf tubes.
5. Add plasmid DNA (final concentration 5 to 20 µg/ml) or proteins (final concentration 10 to 100 µg/ml) and mix. When performing electroporation at 4 °C, precool the cuvettes on ice.
6. Transfer the cell suspension to electroporation cuvettes. Take care that no air bubbles are formed.
7. Electroporation: (settings on the Multiporator®)
   Mode: Eukaryotic cells
   Voltage (U): To enable the optimal voltage to be set on the Multiporator®, it is advisable to perform a series of experiments with several pulse voltages. For adherent cells: between 1 to 5 times the minimum pulse voltage stated in Table 2. For suspension cells: between 1 to 3 times the minimum pulse voltage stated in Table 2.
   Voltages for 2mm cuvette at room temperature:

Cell Diameter [µm]	Voltage	2*Voltage	3*Voltage
8.5	314	628	942

   Time constant (τ): At RT 40 to 100 µs At 4 °C 15 to 40 µs
   Number of pulses (n): 1
8. After pulsing, allow the cell suspension to remain in the cuvette for 5 to 10 minutes. If electroporation was carried out at 4 °C, the cuvettes should be placed on ice for a maximum of 2 minutes after pulsing and should then be incubated in a water bath for 8 minutes at 37 °C.
9. Carefully remove the cell suspension from the cuvette using a Pasteur pipette and cultivate it in 3 to 5 ml culture medium in a 60-mm culture dish. When removing the cell suspension, ensure that the aluminum electrodes are not damaged so that contamination by cytotoxic aluminum ions is prevented.

   Note: After pulsing, the cells should be incubated for 2 to 3 hours at 37 °C before any centrifugation is performed, to ensure resealing of the membrane.

   After the cells have been transferred to the culture medium, they should not be subjected to stress, such as can be caused by shaking or long periods of transport.

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

Each cuvette will contain:
Cells + buffer: 396 µl
Plasmid DNA (500ng/µl): 4 µl

Master mix for 9+1 cuvette (each buffer):
Cells + buffer (OptiMem): 3.96 ml (cells concentration: 1 x 10^6 and 3 x 10^6 cells/ml)
Plasmid DNA (500ng/µl): 40 µl

→Transfer 400 µl from the master mix to each cuvette.

Buffer: OptiMem

Number	1	2	3	4	5	6	7	8	9	10 control
Voltage [v]	314	314	314	628	628	628	942	942	942	628
Pulse length [µs]	40	70	100	40	70	100	40	70	100	70
Number of pulses	1	1	1	1	1	1	1	1	1	1