Electroporation protocol for 3T3 cells

GTporator®

Transfection protocol Protocol No. 09/2008-007

Cell line	3T3 (ATCC CCL-163)
Washing solutions	Phosphate buffered saline (PBS), pH 7.4, GTporator®-M
Cell count	1-3 x 10^6
Electroporation solution	GTporator®-M
Cuvette	2 mm gap width
Volume	80 µl
Temperature	Room temperature
DNA	5 µg in water

Instrument settings

Single amplitude	
Protocol	Square-wave
Voltage	200 V
(Capacitance	960 µF)
Pulse time	20 ms
Pulse number	1

Radio-frequency	
Voltage	230 V
Frequency	40 kHz
Pulses	5 x 4 ms
Interval	1 s
Modulation	100%

1. Trypsinize subconfluent cells
2. Wash the cells once in 1 ml PBS, and once in 300 µl of GTporator® solution
3. Resuspend the cells in 80 µl GTporator® solution with 5 µg of plasmid DNA
4. Transfer into a 2 mm electroporation cuvette
5. Electroporate
6. Immediately add pre-warmed culture medium with serum, transfer the cells into cell culture flask and place in incubator
7. Test the gene expression 24 h after electroporation

Notes:

- The overall incubation time in the GTporator® solution must not exceed 20 minutes to guarantee a successful electroporation!
- The best results are obtained with DNA purified by anion-exchange chromatography.
- DNA should be dissolved in sterile water, using the TE buffer slightly lowers the survival rate.

Typical results:
Survival rate in control cells 60%
Transfection rate: 60-75% with variations depending on the quality of DNA.
Results were measured 24 hours after transfection.

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