# Electroporation protocol for dendritic cells

GTporator®

Transfection protocol Protocol No. 09/2008-005

| | |
|---|---|
| **Cell line** | Primary dendritic cells, Differentiated bone marrow DC |
| **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 | |
|---|---|
| Voltage | 200 V |
| Capacitance | 960 µF |
| Pulse time | 25 ms |

| Radio-frequency | |
|---|---|
| Voltage | 250 V |
| Frequency | 40 kHz |
| Pulses | 5 x 4 ms |
| Interval | 1 s |
| Modulation | 100% |

| Transf. into nucleus | |
|---|---|
| Program | X001 |
| Volume | 120 µl |
| Vector | 2.5-5 µg |

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.



Protean s.r.o., Dolní 2, 370 04 České Budějovice, Czech Republic, Tel.: +420 724 731 831

E-mail: info@protean.cz, web: www.protean.cz