Biontex

DNA-transfection of RAW 264.7 (mouse macrophage-like cell line) using "Biontex K2® Transfection System".

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Materials and Methods

Cell culture

RAW 264.7 cells were cultured in 12-well or 6-well plates with flat bottom (Corning) in high glucose Dulbecco's modified eagle medium (Sigma-Aldrich) supplemented with 0.5 mg/mL penicillin and streptomycin, heat inactivated 10% fetal bovine serum and 2 mM L-glutamine (DMEM/10%FBS). $1x10^5$ cells per well in 1 mL of the medium were seeded in 12-well plates, while $3x10^5$ cells per well in 2 mL of the medium were seeded in 6-well plates 24 hours prior to transfection. At the time of transfection the cell confluence reached 70-80%.

Cell transfection

Cells were treated with K2® Multiplier 2 hours before DNA transfection. For cells cultured in 12-well plates, 16 µl of K2®Multiplier was added to DMEM/10%FBS medium (600 µL total volume) in an eppendorf tube, mixed by pipetting and added gently to cells. Subsequently, 8 µL of K2® Transfection Reagent was mixed with 100 µL of serum-free medium. In a separate tube 2 µg of LPS-free pEGFP-N1 plasmid (Addgene) was diluted to the final volume of 100 µl of the serum-free medium. The DNA solution was added to the solution containing the K2® Transfection reagent (not the other way around) and mixed by pipetting once, followed by 15 minutes incubation at room temperature. The mixture was added dropwise to cell cultures followed by gently swaying the plate. For cells cultured in 6-well plates, volumes of K2®Multiplier solution and DNA mixtures were increased accordingly and are indicated in the table below. Subsequently, cells were cultured for 24 hours and used for experiments. As controls, cells treated with K2®Multiplier only and non-treated cells were used. Transfection efficiency was estimated by fluorescence microscopy.

Dish size	DMEM/10%FBS/ 2mM L-glutamine	K2® Multiplier	K2®Transfection Reagent	DMEM for K2®Transfection Reagent/DNA solution	DNA pEGFP-N1
12-well plate	584 µL	16 µL	8 µL	100 µL/100 µL	2 µg
6-well plate	1176 µL	24 µL	16 µL	200 µL/200 µL	4.5 µg

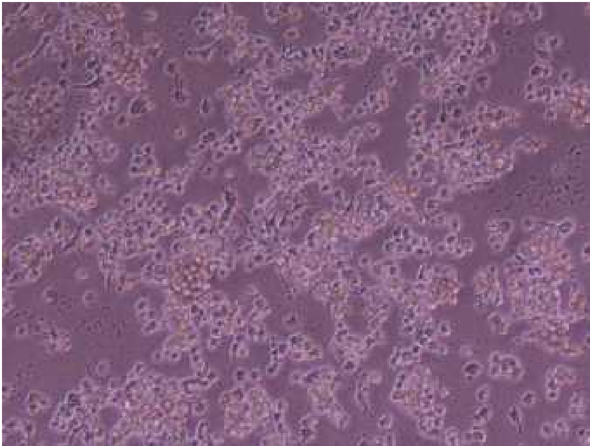
After 24 hours from transfection, cells were collected and seeded in 96-well plates ($1.5x10^5$/well), let to adhere for 2 hours and stimulated with 100 ng/ml LPS from *E. coli* 0111:B4 (ultrapure; List Biological Lab.) in DMEM/10%FBS for 4 hours. The level of TNF-α and chemokine Rantes (CCL5) in culture supernatants was analyzed by ELISA assays according to the manufacturer's instruction (BioLegend).

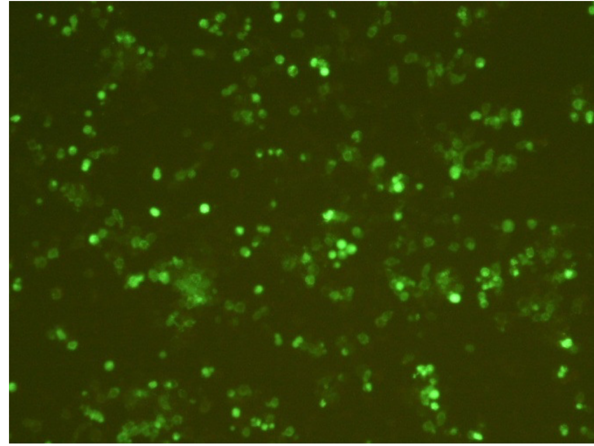
The impact of transfection on membrane fluidity was measured using 1 µM Laurdan (Life Technologies) for labeling of cells in 6-well plate cultures. After labeling (15 min, 37°C), fluorescence of samples was estimated in a microplate reader (Perkin Elmer) using excitation light at 370 nm. The shift of the emission maximum of Laurdan was quantified by the Generalized Polarization (GP) function defined as: $GP=(I_{440}-I_{480})/(I_{440}+I_{480})$, where I_{440} and I_{490} are the emission intensities at 440 and 480nm respectively.

Results

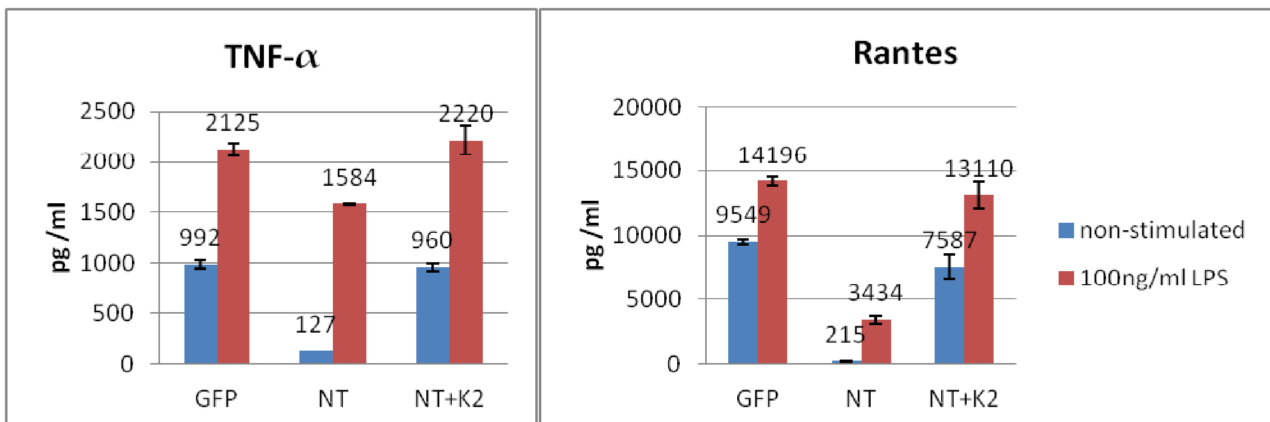
1. Transfection efficiency reached 45-50%.

   Fluorescence microscopy performed 24 hours after adding DNA shows successful transfection of cells with the GFP-bearing plasmid (keeping in mind that RAW 264.7 cells are hard to transfect). Toxicity of "Biontex K2®Transfection System" on RAW267.4 cells was NOT observed.





2. Production of TNF-α and Rantes (CCL5).



GFP – Raw264.7 transfected with plasmid encoding GFP protein
NT – non-transfected Raw264.7 cells
NT+K2 – non-transfected Raw264.7 cells exposed to K2®Multiplier only

3. Measuring plasma membrane fluidity

   With application of the fluorescent probe Laurdan.

   Plasma membrane fluidity of transfected cells was not changed comparing to untransfected cells.