Transfection of RAW 264.7 (Mouse leukaemic monocyte/macrophage cell line) using K2® transfection system

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Materials:

Plasmid: pAAV-GFP (Addgene #32395)

Cells: RAW 264.7 (ATCC Number: TIB-71)

Transfection reagents: K2 Transfection System (Biontex), Fugene (Roche)

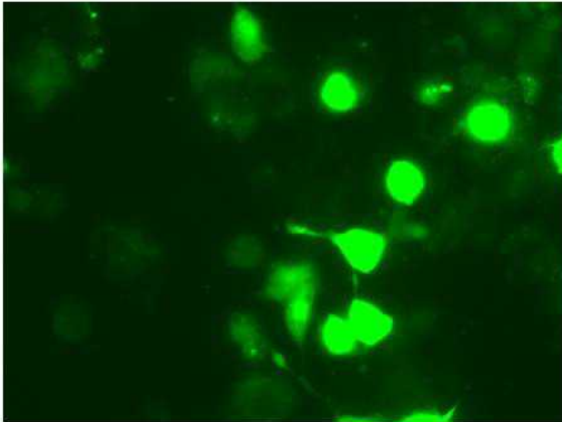
Methods:

RAW 264.7 cells were seeded at 400.000 cells per well of a 6 cm tissue culture dish one day before transfection. For easier fluorescence imaging, cells were seeded on sterile cover slips placed in the wells before addition of the cell suspension. Cells were seeded in 2 ml of medium per well. On the day of transfection, the K2 multiplier (50 µl) was added to 2 ml of medium followed 2 h later by addition of transfection mixes containing plasmid DNA and the K2 transfection reagent. The transfection mix was prepared by diluting 10 µg pAAV-GFP plasmid and 20 µl K2 Reagent in Optimem serum free medium (50 µl Optimen for each, DNA and K2 reagent). These two components were then pipetted into a single microtube, mixed and incubated for 20 min at room temperature. The transfection mix was then added to cells and incubated for another 24 h. In parallel, samples transfected using Fugene were prepared. A standard protocol recommended by the manufacturer was applied: 10 µg plasmid pAAV-GFP plasmid were mixed with 14,1 µl Fugene HD and 225 µl Optimem, and added to the cells followed by incubation for 24 h.

After 24 h of transfection, cells were fixed using 2% formaldehyde (5 min), washed 2x with PBS, and mounted in Dako fluorescent mounting medium on glas slides.

Results:

Transfection using K2
GFP-positive RAW 264.7 cells: 45%



Transfection using Fugene:
GFP-positive RAW 264.7 cells: < 1%