Transfection of adipose derived primary MSC using K2 Transfection System

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Materials:

- DMEM, high glucose, GlutaMAX™ Supplement, HEPES (Gibco)
- Fetal Bovine Serum (Sigma)
- PBS
- Trypsin solution
- Sterile Eppendorf tubes
- 75T cell culture flask (BD bioscience)
- 24-well plate (BD bioscience)
- Plasmid DNA: pE-GFP-C1 (Clontech)

1. Primary human adipose tissue derived Mesenchymal Stem Cells (MSC) were grown in DMEM supplemented with 15% FBS and 1% penicillin-streptomycin it T75 cell culture flask. Cells were trypsinized after 80% confluence and seeded on 24-well plate in density $10^4/cm^2$ ($2x10^4$ per well in 0,5 ml DMEM).
2. Next day K2 multiplier (5µl) was added to cells 2h prior transfection. Transfection mixtures were prepared 20 minutes prior transfection according to Table I and following protocol:

Table I.

DNA:K2 ratio- **1:2**
Plate: **24-well plate**
Amount of medium per well: **0,5ml**
K2 multiplier: 10µl, 2h incubation

DNA:K2 ratio								
1:2			**1:3**			**1:4**		
DNA [µg]	**K2 [µl]**	**DMEM [µl]**	**DNA [µg]**	**K2 [µl]**	**DMEM [µl]**	**DNA [µg]**	**K2 [µl]**	**DMEM [µl]**
0,2	0,4	30+30	0,2	0,6	30+30	0,2	0,8	30+30
0,4	0,8	30+30	0,4	1,2	30+30	0,4	1,6	30+30
0,6	1,2	30+30	0,6	1,8	30+30	0,6	2,4	30+30

The indicated amount of K2 transfection reagent (See Table I) was added to 30 µl of serum-free cell culture medium (DMEM) in a 1.5 ml Eppendorf centrifuge tube. In a separate tube indicated amount of the plasmid DNA (pE-GFP-C1) was mixed with 30 µl of serum-free medium. The media containing K2 and DNA were combined and mixed by pipetting up and down 5 times. The tubes were incubated at room temperature for 20 min for lipid-DNA complexes to form. At the end of the incubation time, the lipid-DNA complex mixtures (60 µl per well) were pipetted onto the cells, mixed by tilting the plates a few times and thereafter incubated at 37°C incubator under 5% CO2. After 24h cells were trypsinised analyzed by flow cytometry.

Results

Best efficiency (30%) was obtained using 1:4 ratio and 0,6µg of DNA per well (Figure 1).

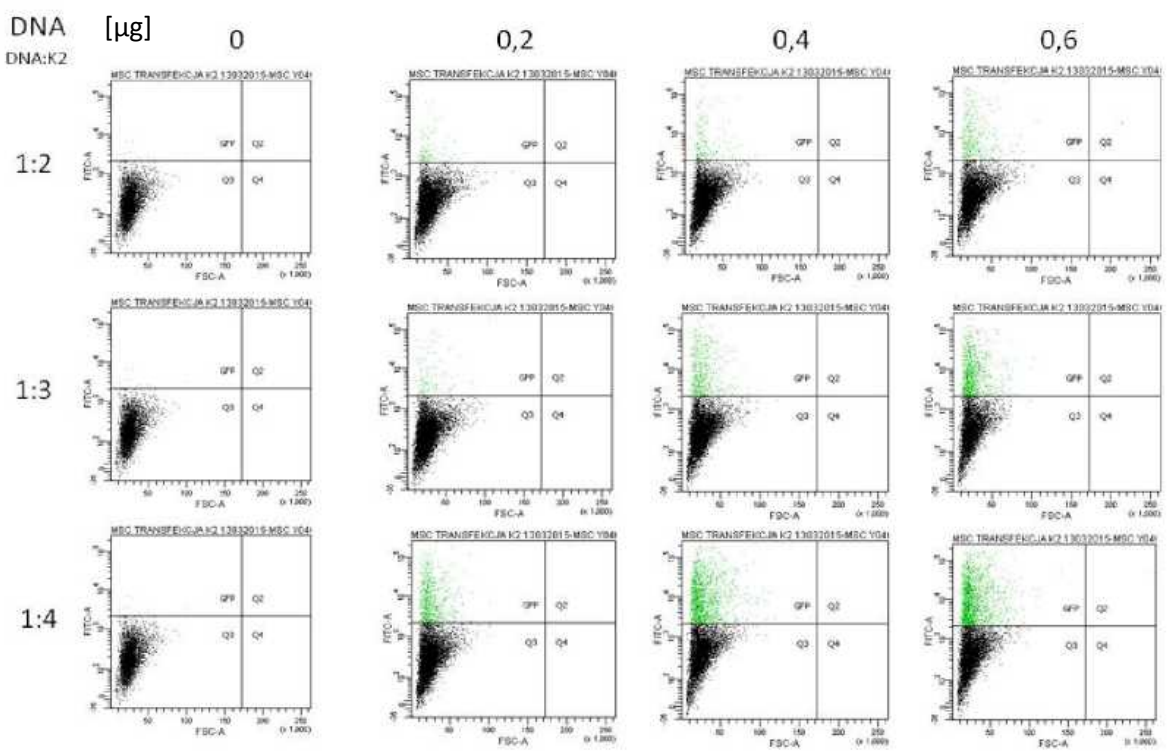

Figure 1. GFP expression in MSC cells after transfection with different K2:DNA ratio and DNA amount.