Comparison of K2 Transfection System with Lipofectamine 2000 (Invitrogen) for the transient transfection of HEK293T cells.

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Materials and Methods

Reagents - K2 Transfection System reagents were stored and handled according to the manufacturer's instructions.

Plasmids - An *E. coli* XL1-Blue LB culture was used to amplify the pcDNA3.1-hCD4 plasmid, which contains the human CD4 membrane glycoprotein, and purified with a Wizard plus SV miniprep kit (Promega). The plasmid was eluted in nuclease-free H_2O (Promega).

Cell culture - HEK293T cells were cultivated in DMEM (Invitrogen) supplemented with 10% HyClone fetal bovine serum (Thermo Scientific), 10 mM HEPES (Invitrogen) and passaged three times a week in 75 cm^2 culture flasks (Falcon).

Transfection - 8×10^5 cells were seeded per well of a 6-well plate (Costar) in 2 ml DMEM (Invitrogen) supplemented with 10% HyClone fetal bovine serum (Thermo Scientific), 1 mM sodium pyruvate (Invitrogen) and 0.075% (m/v) $NaHCO_3$ (Invitrogen). After overnight incubation at 37°C and 5% CO_2, the medium in each well was aspirated and carefully replaced with 2 ml fresh medium. General confluency at this point is 50%.

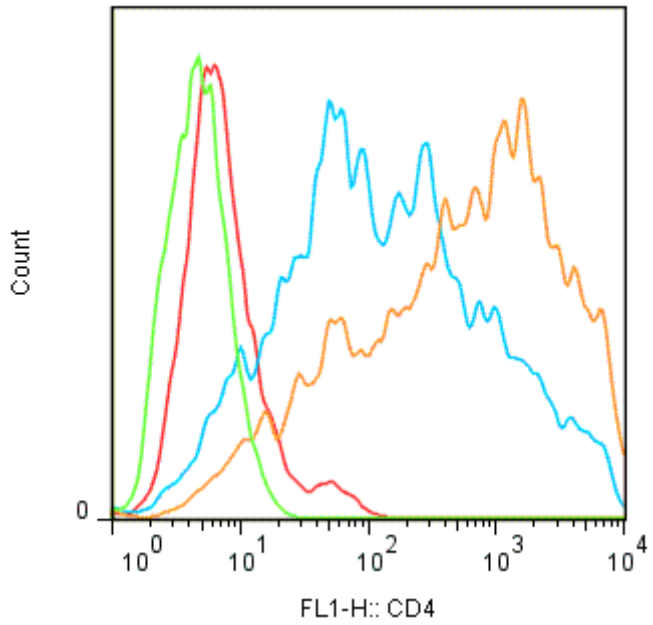
a) For K2 transfection, 20 µl K2 Multiplier was dropped on top of the medium, mixed by gentle swirling, and incubated for 2 hours at 37°C and 5% CO_2. Next, 2.5 µg plasmid DNA and 9.6 µl K2 Transfection reagent were both diluted in separate polypropylene tubes (BD) filled with 120 µl serum-free DMEM. The solutions were combined and mixed by pipetting up- and down once. The mixture was incubated at room temperature for 15 minutes, drop-wise added to the cells and mixed by swirling the plate.
b) The cells for Lipofectamine transfection were incubated in the fresh medium at 37°C and 5% CO_2 one hour before transfection. 2.5 µg plasmid DNA and 9.5 µl Lipofectamine 2000 were both diluted in separate polypropylene tubes (BD) filled with 100 µl serum-free DMEM. The solutions were combined and mixed by pipetting up- and down once. The mixture was incubated at room temperature for 5 minutes, drop-wise added to the cells and mixed by swirling the plate.

After a 6 hour incubation at 37°C and 5% CO_2, cells were resuspended and transferred to 5 ml polypropylene tubes and centrifugated at 400 ×*g* for 5 minutes. Medium was replaced with 1.3 ml fresh medium and cells were resuspended before seeding 100 µl of the cell suspension per well of a 96-well plate (Corning). Protein expression was measured after 24 hour incubation at 37°C and 5% CO_2.

Cell staining - Cells were resuspended from the 96-well plate, washed in cold PBS and stained with anti-human CD4-FITC (Clone SK3, BioLegend). Expression of CD4 was determined on a BD FACSCalibur flowcytometer. 10,000 cells were collected for analysis.

Results

The mean fluorescence intensity (MFI) and geometric mean fluorescence intensity (GMFI) of each sample was calculated with FlowJo software. Transfection efficiency was determined through gating based on the non-transfected, unstained cell population.



	Sample	MFI	GMFI	Transfection efficiency
	Not Transfected, unstained	5	5	-
	Not Transfected, stained	10	7	-
	Lipofectamine	582	132	77%
	K2 Transfection Reagent	1369	434	89%

Conclusion

K2 Transfection Reagent produces a significantly higher transfection efficiency of HEK293T cells compared to Lipofectamine 2000 (89 vs 77%). The average expression is also higher, which can be clearly seen on the flowcytometry histogram (MFI: 1369 vs 582).