Evaluation of siRNA transfection efficiency of K2® transfection system in A2780 and A2780cis ovarian cancer cells

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Materials and Methods

Cell culture

The ovarian carcinoma cell line A2780 and the cisplatin-resistant variant A2780cis (European Collection of Cell Cultures, United Kingdom) were cultivated as monolayers in RPMI-1640® medium supplemented with 10% fetal calf serum (FCS), 100 U/mL penicillin and 0.1 mg/mL streptomycin (37 °C, 5% CO_2). Prior to transfection cells were seeded in 96-well plates at 1 x 10^4 , 2 x 10^4 and 3 x 10^4 cells per well in 90 µL RPMI 1640 without antibiotics and grown overnight. After transfection with fluorescent siRNA (siGLO, ThermoScientific), cells were analyzed by fluorescence measurement (Fluoroskan Ascent) to evaluate transfection efficiency. Cell viability was assessed by MTT assay (Multiskan Ascent).

Cell transfection

(Please find accurate K2® reagent amounts in the table below)

Cells were treated with 1.4 µL K2® multiplier two hours before siRNA transfection. By gently swaying the plates the multiplier was mixed with the medium. The siRNA was diluted in RPMI 1640 medium without FCS and antibiotics. This dilution was added to the dilution of K2 transfection reagent in RPMI1640 medium leading to a final siRNA concentration of 50 nM. By pipetting once the solutions were mixed and then incubated for 15 minutes at room temperature. Each well was supplemented with 10 µL of the mixture. Again, plates were gently swayed to assure a uniform distribution of the transfection reagent.

		In 96-well plate	**for 12 wells**				
			A	**C**	**E**	**G**	
1.	Mix	RPMI 1640 w/o FCS and AB	75	75	75	75	µL
		K2	3	6	9	12	µL
2.	Mix	RPMI 1640 w/o FCS and AB	75	75	75	75	µL
		siRNA (10µM)	7,5	7,5	7,5	7,5	µL
3.	Mix	Diluted siRNA	75	75	75	75	µL
		Diluted K2	75	75	75	75	µL
Add 10 µL of 'Mix #3' to each well							
		K2/well	0.25	0.5	0.75	1.0	µL

Results

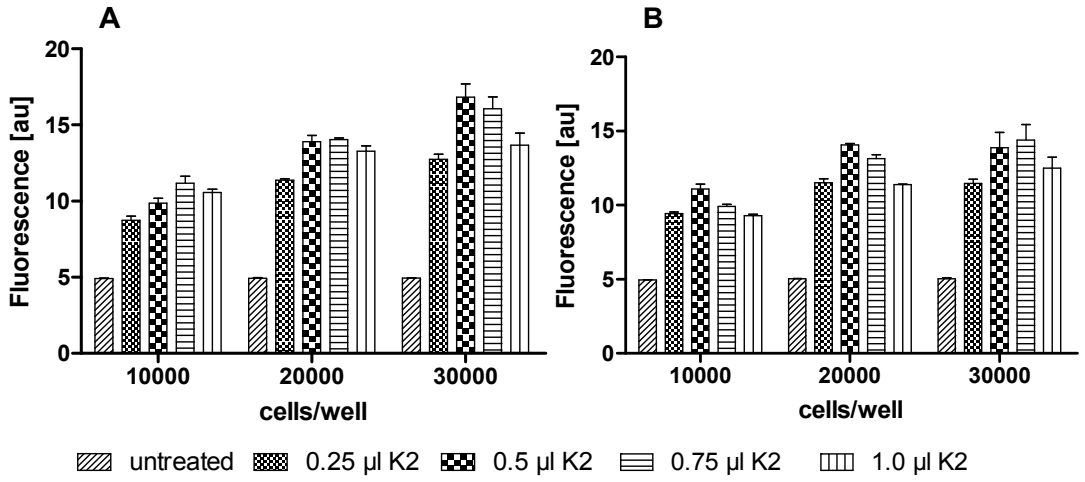

Figure 1 - Fluorescence after siGLO® transfection with K2® in (A) A2780 and (B) A2780cis cells (n=3)

With an increasing amount of K2®, the transfection efficiency could be increased dependent on cell number. After 24h incubation highest fluorescence could be measured after transfection with 0.5 µL K2/well to 0.75 µL K2/well in A2780 and A2780cis cells.

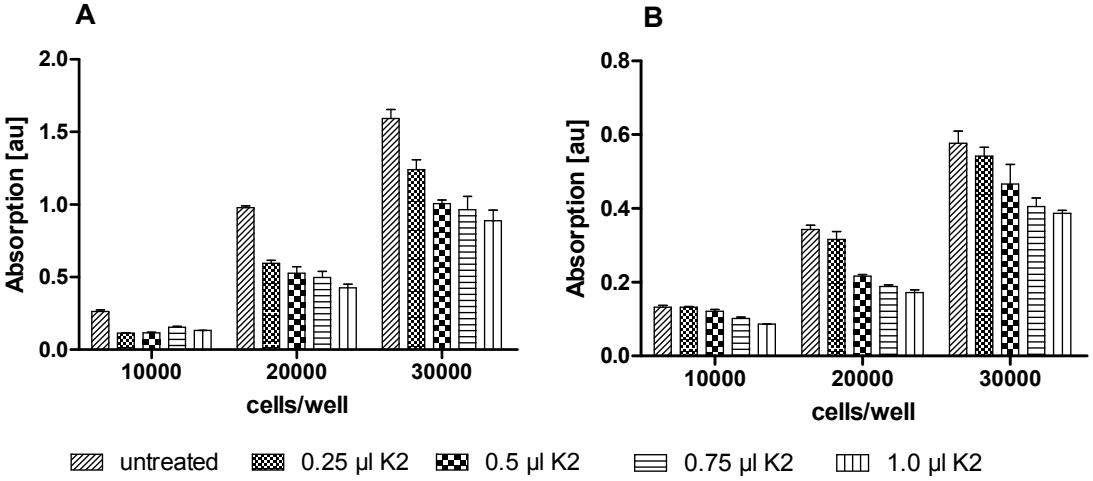

Figure 2 - Viability of (A) A2780 and (B) A2780cis cells after transfection of siGLO® with K2® transfection system (n=3)

Cell viability after transfection with different amount of K2® was evaluated using an MTT-based assay. The absorption values, which are proportional to the number of viable cells, decrease with increasing K2® concentrations. The results show that the viability of both cell lines decreases after transfection in a concentration-dependent manner.

Conclusions

The K2® transfection system was successfully applied for transfecting A2780 and A2780cis cells with a fluorescent siRNA. Cell viability is influenced by the transfection as the proliferation rate is reduced compared to untreated control cells. We consider 0.5 µL/well the optimal concentration of K2®. Cells show a high transfection rate while they are still viable.