A comparison of DNA-transfection of Hela Cells between using "Biontex K2® Transfection System" and "Lipofectamine®2000 DNA Transfection Reagent"

Jiawen Wang, Guiqing Xiao, School of Biomedical Sciences, Huaqiao University, Quanzhou, Fujian, China

Materials and Methods

Cell culture

Hela cells were seeded at 15000/100 µl per well in 96-well plates (NEST Biotechnology) in antibiotic-free Gibco® RPMI-1640 medium containing 10% fetal bovine serum. Transfection was performed when cells had reached a confluency of 90-100%.

Cell transfection using Biontex K2® Transfection System

Cells were treated with K2® Multiplier, 2 hours before DNA transfection. K2® Transfection Reagent was mixed with serum-free medium (Gibco® RPMI 1640) and left on room temperature during preparation of the DNA. Plasmid-DNA (psiCHECK™-2) encoding *hluc+* and *hRluc* was mixed with serum-free medium. DNA solution and the solution containing K2® Transfection reagent were mixed by gently pipetting up and down, followed by 15 minutes incubation at room temperature. DNA-lipid complex was applied to cells by slow dropwise addition to the medium followed by gently swaying the plate to achieve mixing, and incubated at 37C and 5% CO_2. 24 h later the transfection mixture was removed and replaced with fresh complete growth medium. After incubating for another 24h, a dual luciferase assay was performed.

Cell transfection using Lipofectamine®2000 DNA Transfection Reagent

Both Lipofectamine® Reagent and Plasmid-DNA (psiCHECK™-2) were diluted in Opti-MEM® Medium (Life technology) and incubated for 5min. Then they were mixed by gently pipetting up and down, and incubated on room temperature for 20min. After that the DNA-lipid complex were added to the cells. After incubating at 37C and 5% CO_2 for 5h, the transfection mixture was removed and replaced with fresh complete growth medium. After incubating for another 24h, a dual luciferase assay was performed.

Method	Plasmid-DNA per well	Transfection Reagent per well	Solutions used for dilution, Transfection reagent/DNA
Biontex K2® Transfection System	150ng	0.6µl	RPMI 1640 medium□ 5µl/5µl
Lipofectamine®2000 DNA Transfection Reagent	100ng	0.4µl	Opti-MEM® Medium□ 25µl/25µl

Results

Three independent experiments were performed and the results are shown below.

1	Negative control	Lipofectamine®2000 DNA Transfection Reagent	Biontex K2® Transfection System
hluc+	826	17376	48437
	839	22820	59899
	674	20246	128735
	627	25793	167177
	577	21217	122899
	756	28145	183163
	623	17676	137071
	607	19018	111069
hRluc	3485	214838	2097664
	2965	270495	1900636
	4122	221748	4282202
	2148	270071	4554676
	2157	272700	4467761
	2482	353192	5242547
	2027	209215	5197431
	1994	223415	4839713

2	Negative control	Lipofectamine®2000 DNA Transfection Reagent	Biontex K2® Transfection System
hluc+	263	41469	92181
	194	15621	135792
	190	21644	141191
	193	13607	117157
	564	24524	151923
	205	26004	295269
	220	22282	147494
	443	20068	145950
hRluc	1729	347642	3127495
	1465	158124	4290304
	2193	246636	4488200
	1764	172929	2720256
	2418	217878	3918058
	1768	260599	8388204
	1789	218304	6240780
	1818	183865	5122873

3	Negative control	Lipofectamine®2000 DNA Transfection Reagent	Biontex K2® Transfection System
hluc+	1967	10737	17374
	662	11395	27702
	562	5977	15092
	610	16976	22415
	596	9013	7855
	610	5958	7456
	630	3784	4089
	612	2296	1937
hRluc	3604	95398	909847
	2226	76344	664901
	2066	46475	423355
	1977	146201	581109
	1979	75161	192670
	1984	60485	158750
	1873	30749	86275
	1895	19538	48866

Conclusions

Plasmid-DNA□psiCHECK™-2 encoding *hluc*+ and *hRluc* was transfected into Hela Cells using K2® Transfection System and Lipofectamine®2000 DNA Transfection Reagent respectively. According to the dual luciferase assay the K2® Transfection System had outperformed Lipofectamine®2000 DNA Transfection Reagent under current experimental conditions.