DNA-transfection of human embryonic kidney cells 293 (HEK293) using "Biontex K2® Transfection System"

1) Alessandra Zevini, University "La Sapienza", Department SAIMLAL, Via A. Scarpa 14, 00161 Rome, Italy
2) Federica Campolo, PhD, Department of Experimental Medicine University "La Sapienza" Viale Regina Elena 324 00161 Rome, Italy
3) Prof. Fabio Naro, MD PhD, University "La Sapienza", Department SAIMLAL, Via A. Scarpa 14, 00161 Rome, Italy

Cell culture

$1x10^5$ HEK293 cells were seeded the day before the transfection in a 24 well plate (Sarstedt) in high glucose Dulbecco's modified eagle medium (Euroclone) containing 10% fetal bovine serum. The amount of medium for each well was 0,5 ml. Transfection was performed when cells reached a confluence of 70-80%.

Cell transfection

Cells were treated with K2 Multiplier 2 hours before DNA transfection. K2 Transfection Reagent was mixed with 30 µl of Opti-MEM (Life Technologies) and incubated for 5 minutes at room temperature. Plasmid-DNA encoding EGFP was mixed with 30 µl of Opti-MEM. DNA solution was added to the solution containing the K2 Transfection reagent and incubated for 20 minutes at room temperature. Transfection solution was applied to cells by slow dropwise addition to the medium. Transfections were incubated at 37°C and 5% CO2 for 24 or 48 hours.

To assess the efficiency of the K2 Transfection System five conditions were tested varying K2 Multiplier, K2 Transfection Reagent or DNA amounts:

	K2 MULTIPLIER (µl)	K2 TRANSFECTION REAGENT (µl)	DNA (µg)
1)	15	4	1
2)	5	2,4	0,6
3)	15	2,4	0,6
4)	10	4	1
5)	10	2,4	0,6

Results

Fluorescence microscopy analysis after 24 or 48 hours shows successful transfection with different transfection rates. Among the examined conditions, 5) seems to be the most efficient with > 80% of transfected cells after 24 hours from transfection (see image below). Cells show a perfectly healthy morphology excluding any cytotoxic effects of the transfection system.