K2 Transfection reagent – Technical Note

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Cell line	*Human Neuronal SH-SY5Y*
Plasmid	*pEGFP-NI (Clontech) expressing EGFP*
Materials	*Sterile 24-well tissue culture plates*
	75 ml cell culture flasks
	Sterile Eppendorf tubes
	Trypsin + EDTA solution
	DMEM high pyruvate + 10 % fetal bovine serum
Trasfection Reagents	*K2 Trasfection System (Biontex)*
	Lipofectamine 2000 (Life Technologies)
	Lipofectamine 3000 (Life Technologies)
Seeding	*70.000 cells/well*
Protocol	Day after seeding, the DMEM complete medium was replaced with 1 mL of fresh complete medium, containing 9.5 µL of K2 Multipler/mL of medium; then, the plate was incubated at 37°C for 2h. Transfection was performed as follow: - 2 µL of plasmidic DNA (250 ng/µL) were diluted in 28,5 µL of serum-free DMEM; - 2 µL of K2 Transfection reagent were diluted in 28,5 µL of serum-free DMEM; - Samples were mixed and incubated at RT for 20 mins; - Transfection mix was directly added in each well. Plate was incubated for 24h. In parallel, the cells were transfected with same amount of plasmid DNA using Lipofectamine 2000 and Lipofectamine 3000 (Life Technologies), according with manufacture's protocol.
Cell viability results	Lipofectamine 2000 shows the most pronounced toxic effect compared to the other reagents as showed in fig 1A. K2 reagent results less toxic than the previous (fig 1B) while Lipofectamine 3000 seems to have the less toxic effect on cells, as showed in fig 1C. Cell transfected with both K2 and Lipofectamine 3000 mantain a healthy cell morphology.
Efficiency of transfection	K2 reagent show a high efficiency with an estimated value of 80-90% of transfected cells, as showed in fig 2B. Fig 2A and 2C shows respectively

	samples treated with Lipofectamine 2000 and 3000.
Notes	Improved efficiency of trasfection, showed by K2 reagents, could be directly related to the less toxic effect of this reagent compared the others.