DNA transfection with K2® Transfection System in human Hepatic Stellate cell line LX-2

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Materials and Metods

Human hepatic stellate cells LX-2 were thawed in DMEM High Glucose, 10% FBS, 2mM Glutamine and then expanded in 2% FBS. Cells were seeded 24h before transfection in triplicates in a 12-well plate, in order to reach 80-90% confluency at the time of transfection.
All reagents were brought to room temperature (RT) before transfection.Two hours before transfection, culture medium was changed with complete medium w/o antibiotics and K2® Multiplier was added, as reported in Table 1. Cells were transfected with pCMV-EGFP (pEGFP-N1, Clontech). Efficiency of the K2® Transfection System was compared to that of Promega FuGENE® HD Transfection Reagent. The same quantity of plasmid DNA at the same ratio DNA/Transfection Reagent (1:4) was used for both transfection systems.

For the K2® Transfection System protocol, DNA was resuspended in Optimem® medium, as follows:
SOLUTION A: 50 µl Optimem® + 1.33 µg DNA
SOLUTION B: 50 µl Optimem® + 5.3 µl K2® Transfection Reagent

Solution A and B were combined and gently mixed by pipetting up and down once. DNA-lipid complexes were incubated for 15 minutes at room temperature before adding them to the cells. Medium cell culture was replaced with complete medium 24 hours post-transfection.

Table1. Parameters of DNA transfection with K2® Transfection System used in the test.

Dish size	DMEM (ml)	K2® Multiplier (µl)	K2®Transfection Reagent (µl)	Volume for K2®Transfection Reagent (µl)	Volume for DNA dilution (µl)	Approx.tot volume (µl)
12 well	870	25	5.3	50	50	1000

Total RNA was extracted with ReliaPrep™ RNA Tissue Miniprep System according to manufacturer protocol at 48 hours post-transfection. The expression of EGFP was determined using SYBR Green quantitative Real-Time PCR with MiniOpticon real-time detection System and anaysed with CFX Manager software (Biorad). EGFP mRNA levels were normalized to beta-actin levels.

Results

As reported in Figure 1, K2® Transfection System showed superior transfection efficiency compared to FuGENE® HD Transfection Reagent. No cytotoxic effects were observed.

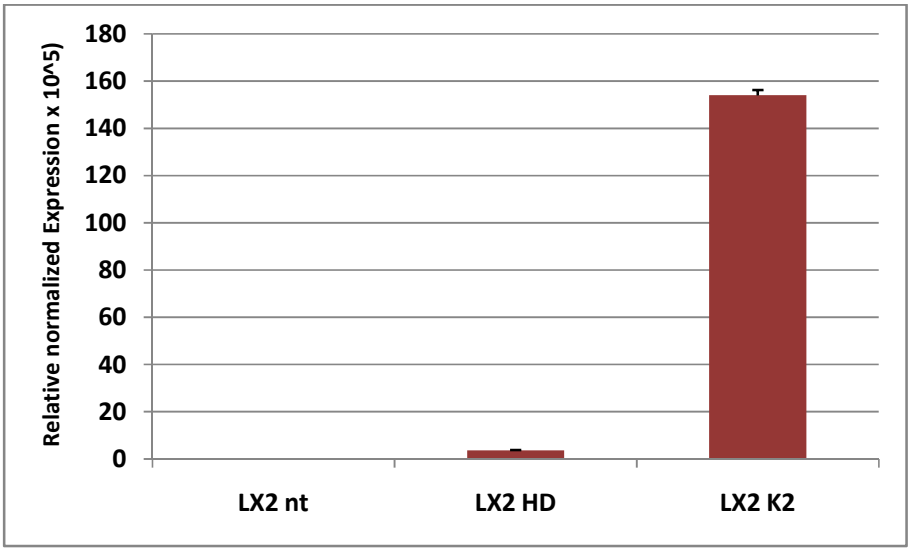

Figure1. qPCR analysis showing the EGFP expression levels normalized to housekeeping mRNA beta-actin in LX-2 cells, at 48 hours post-transfection.