DNA transfection with K2® Transfection System in human Hepatocarcinoma cell lines HepG2 and Hep3B.

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Materials and methods.

HepG2 and Hep3B cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10% FBS and 2mM Glutamine, and 24 hours before transfection 1.5 x10^5 cells were plated in each well of a 48-well dish in 0.25 ml of culture medium. At the day of transfection, all reagents were brought to room temperature (RT). Two hours before transfection culture medium was changed with complete medium w/o antibiotics and K2® Multiplier was added, as reported in Table1. HepG2 cells were transfected with pCMV-EGFP (pEGFP-N1, Clontech) and efficiency of the K2® Transfection System was compared to that of Promega FuGENE® HD Transfection Reagent. Transfection efficiencies of K2® Transfection System was compared to Promega FuGENE® HD and Lipofectamine® LTX with Plus™ Reagent for Hep3B cells. The same quantity of plasmid DNA at the same ratio DNA/Transfection Reagent (1:4) was used for all transfection systems.

For the K2® Transfection System protocol, DNA was resuspended in Optimem® medium, as follows:
SOLUTION A: 15 µl Optimem® + 0.4 µg DNA
SOLUTION B: 15 µl Optimem® + 1.6 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)
48 well	210	7.5	1.6	15	15	250

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 analysed with CFX Manager software (Biorad). EGFP mRNA levels were normalized to the mRNA levels of human ribosomal protein L34. Flow-cytometric analysis with BD FACSCalibur™ was also performed in Hep3B cells.

Results for HepG2 cell line.

As reported in Figure 1, the K2® transfection System resulted in the highest transfection efficiency, when compared to FuGENE® HD system.

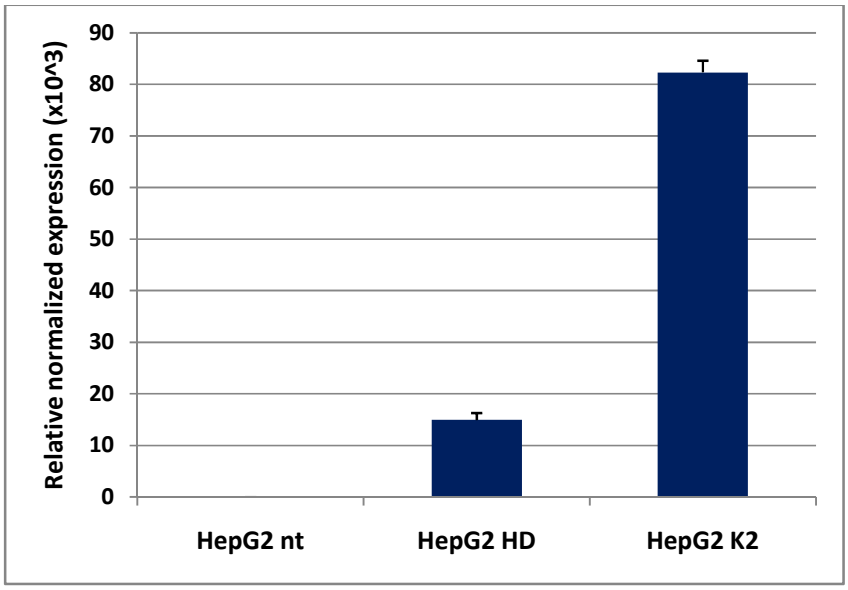


Figure1. qPCR analysis showing the EGFP expression levels normalized to housekeeping mRNA L34 in HepG2 cells, at 48 hours post-transfection.

Results for Hep3B cell line.

Hep3B cells are difficult to transfect at high efficiency. To evaluate the performance of the three transfection reagents in this cell line, qPCR analysis was performed to measure the mean EGFP expression levels (Figure 2).

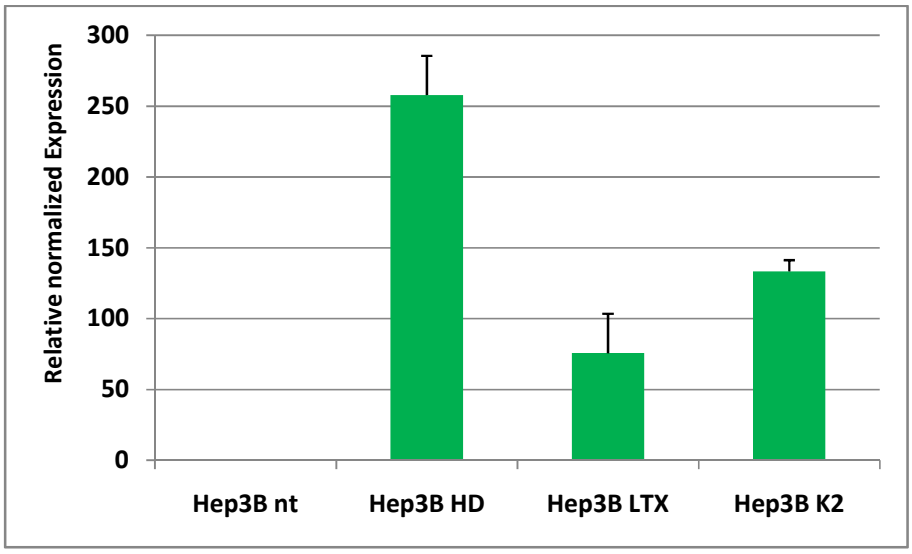
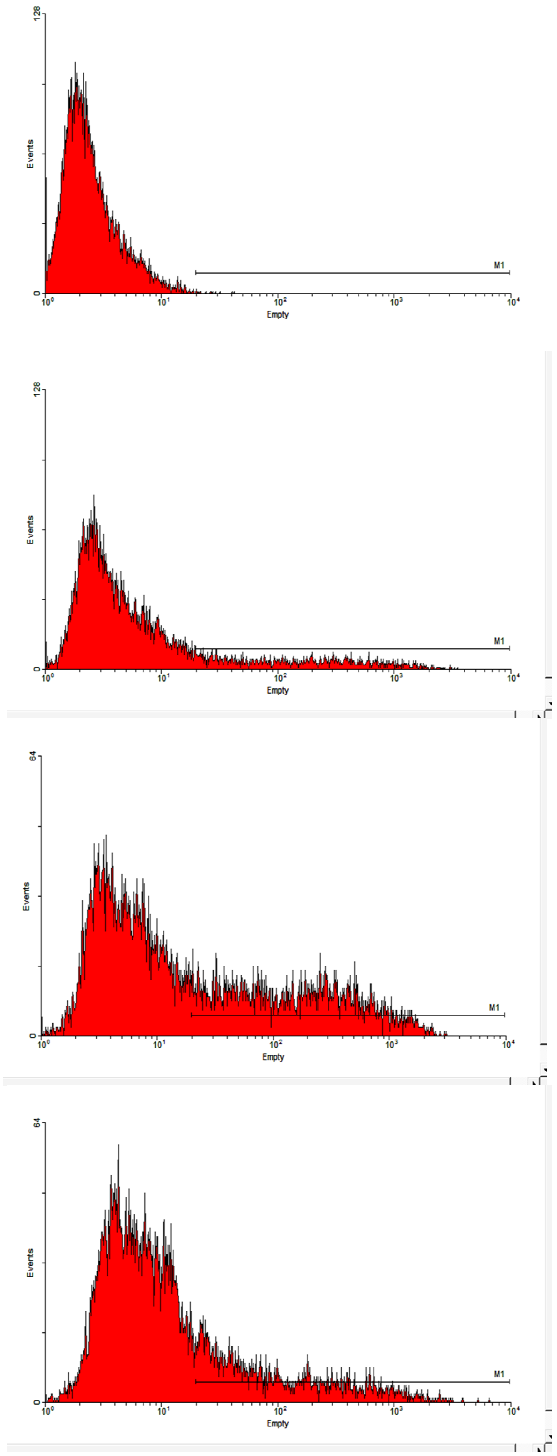


Figure 2: qPCR analysis showing the EGFP expression levels normalized to housekeeping mRNA L34 in Hep3B cells, at 48 hours post-transfection.

Flow-cytometric analysis was performed to measure differences in the intensity of EGFP positivity, which can be related to the number of plasmid copies incorporated by the cells. As shown in Figure 3, the three transfection reagents differered in the percentage of highly EGFP+ cells (signal intensity between 102 and 103), indicating that FuGENE® HD system and K2® transfection Systems deliver more copies of pCMV-EGFP plasmid per cell, compared to Lipofectamine® LTX.



Not transfected
(0.8%)

LTX
(16%)

HD
(40%)

K2
(25.5%)

Figure 3. Flow-cytometric analysis showing the distribution of EGFP+ cells in Hep3B transfected with pCMV-EGFP.

Conclusion

K2® transfection system was compared with other common transfection reagents in two human hepatocarcinoma cell lines, HepG2 and Hep3B. The K2® reagent showed high transfection efficiency in HepG2 cells. Concerning Hep3B cells, the performance of K2® transfection system was superior to that of Lipofectamine® LTX, especially in terms of plasmid copies incorporated per cell, although less efficient than FuGENE® HD. In this test a unique transfection protocol was used for both cell lines. The transfection efficiency could be optimized for Hep3B cell line, i.e. by slightly changing the DNA/K2 ratio. Most importantly, the K2® transfection system showed no evident effect on cell viability in both HCC lines.