DNA-transfection of human gastric cancer cells line MKN28 using "Biontex K2® Transfection System".

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Materials and Methods

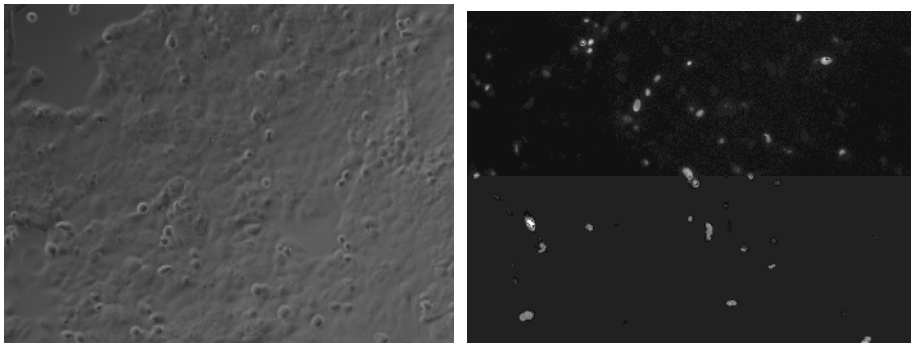
Cell culture

human gastric cancer cells line MKN28 were cultured in 100mm culture dishes, in RPMI 1640 Medium containing 10% fetal calf serum to near confluency and trypsinated thereafter.

Cell transfection

24 hours before transfection, MKN28 cells were seeded in 500µl cell culture medium in 24-well plates at a density of $7x10^4$ cells per well. 5, 10, or 15 µl of K2® Multiplier was added to each well and the plate was returned to the incubator. The transfection mix was prepared after 2 hours of incubation with K2® Multiplier. 0.5 µg pEGFP-PHF8 plasmid DNA was added to 25 µl Opti-MEM® in a 1.5 ml Eppendorf centrifuge tube. In a second tube, 1.5 µl K2 Transfection Reagent was added to 25 µl of Opti-MEM®. Both tubes were mixed by gentle tapping the tube. The solutions of the tubes were combined and mixed as described above. The tube was allowed to stand at room temperature for 20 min. After incubation the solution (50 µl/well) was added dropwise to the cells. The transfection mix was distributed in the well by gently shaking the plate horizontally. The cells were placed back to the incubator. After 48 hours the transfection efficiency was monitored by fluorescence microscopy (Figure).

A B



C D

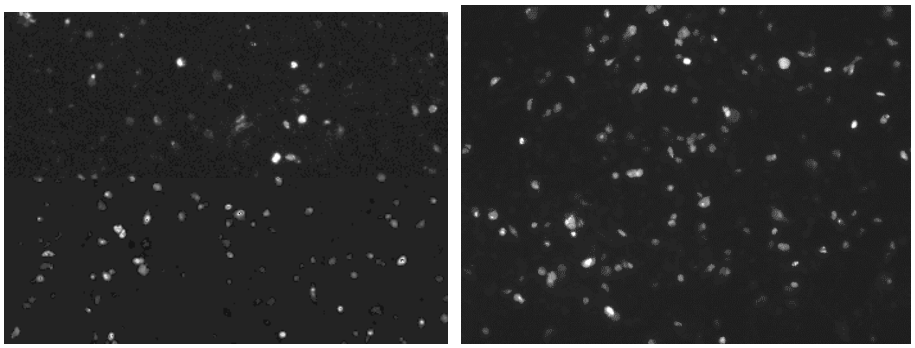


Figure: MKN28 cells were transfected with pEGFP-PHF8 using K2® Transfection Reagent as described above. Un-transfected MKN28 (A), 5µl of K2® Multiplier (B), 10µl of K2® Multiplier (C), and 15µl of K2® Multiplier (D).