DNA-transfection of human gastric adenocarcinoma cell line (MKN28) using "Biontex K2® Transfection System".

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Materials and Methods

Cell culture

One day before transfection, plate 250.000 cells (MKN28) in a single well of a 6-well dish in 2mL of Dulbecco's modified eagle medium (DMEM) without antibiotics containing 10% FBS. Incubate the cells for 24h at 37°C in a 5% CO2 atmosphere.

Cell transfection

2 hours before DNA transfection, cells were treated with 45 µL of K2® Multiplier. Two plasmid-DNA encoding gastrokine 1 (GKN1) (protein involved in gastric cancer but absent in gastric cancer cells) and GFP (green fluorescent protein) were co-trasfected into MKN28 cells.
For each transfection samples, prepare two solution:
A- 135 µL serum-free medium
3.2 µg DNA (1.6 µg EGFP and 1.6 µg pcDNA3.1-GKN1)
B- 135 µL serum-free medium
10.8 µL K2® Transfection reagent
Mix each solution by gently pipetting up and down once.
DNA solution was added to the solution containing the K2® Transfection reagent (not the other way around) and mixed by inverting the tubes, followed by 20 minutes incubation at room temperature. Transfection solution was applied to the cells, mix gently by agitating the cell culture vessel and incubate at 37°C in a 5% CO2 atmosphere for 24 hours.

Culture Vessel	Well size	Volume of DMEM	K2® Multiplier	K2® Transfection reagent	DNA (µg)
6-well	35mm	2mL	45 µL	10.8 µL	3.2 µg

Results

After co-transfection, MKN28 cells were analyzed at optical microscope. Transfection efficiency was estimated by evaluating the fluorescence image of the EGFP (Fig.1).
Western blot analysis was used to evaluate the expression level of GKN1 (Fig. 2).

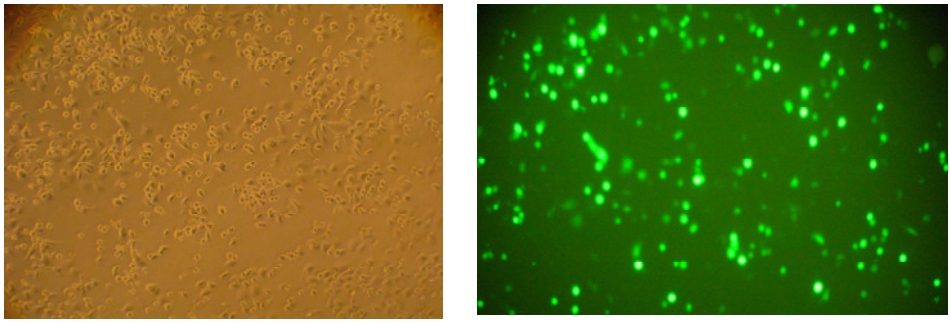

Fig. 1. MKN28 cells after transfection with EGFP vector. Light image (left); fluorescent image (right).

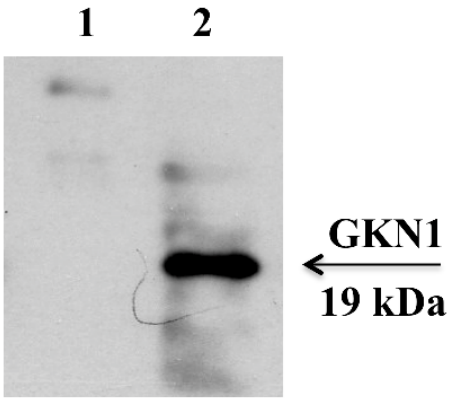

Fig. 2. Western blot of GKN1 expression in MKN28 cells. 1, cell extract of untransfected MKN28 cells; 2, cell extract of MKN28 cells transfected with pcDNA3.1-GKN1.

Conclusions

K2 transfection system shows high transfection rates (70–80%) as illustrated in Fig1. Furthermore MKN28 cells have a mortality rate lowest than other transfection system and remained perfectly healthy after transfection.