DNA-transfection of human SMMC-7721 liver cancer cells using "Biontex K2® transfection system

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Materials and Methods

Cell culture

SMMC-7721 cells were cultured in 6 well plates (Nunc) in high glucose Dulbecco's modified eagle medium containing 10% fetal bovine serum (Gibco) without antibiotics. The volume of medium was 1ml per well. Transfection was performed when cells had reached a confluence of 50-60%.

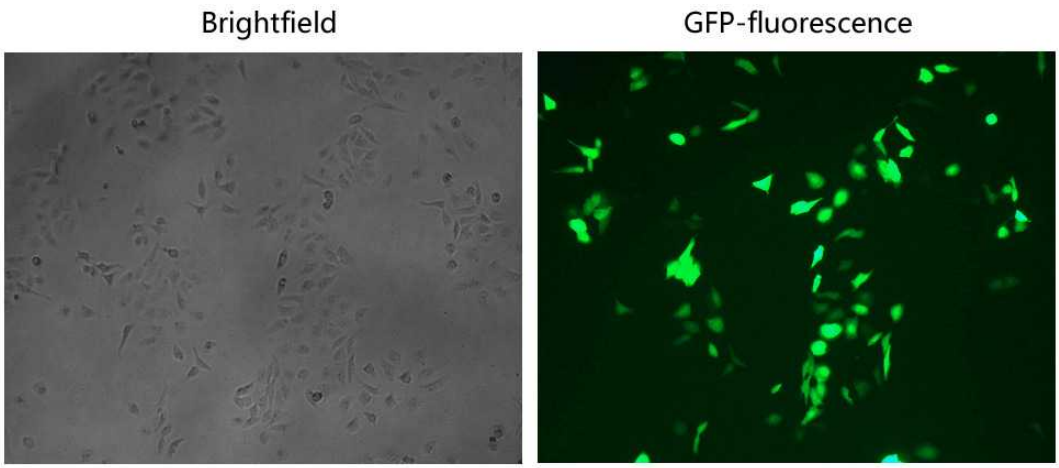
Cell transfection

Cells were treated with K2 Multiplier, 2 hours before DNA transfection. K2 Transfection Reagent was mixed with Opti-MEM Reduced Serum Medium (life technologies) and left at room temperature during preparation of the DNA. Plasmid-DNA pMAXGFP encoding GFP were diluted into serum-free Opti-MEM medium. DNA solution was mixed with Opti-MEM containing K2 Transfection Reagent, followed by incubation for 20 minutes at room temperature. Transfection solution was then added to cells dropwise to the medium followed by gently swirling. Cells were incubated at 37ºC in a humidified incubator containing 5% CO2 for 48 hours. Transfection efficiency was estimated under an inverted fluorescence microscopy.

Table. Parameters used for plasmid DNA transfection for 6-well-format

Culture Vessel	DMEM	K2 Multiplier	K2® Transfection reagent	Opti-MEM Transfection reagent/DNA	pMAX-GFP plasmid
6 well plate	2ml	30ul	10ul	120ul/120ul	2.4ug

Results



Conclusion

1. Transfection efficiency: an approximate transfection efficiency of 50% can be achieved when cells were in the range of 50-90% confluency.

2. Toxicity: No observable morphological changes were found during the transfection experiment.