Transient DNA-transfection of human pancreatic BxPC-3 cells using "Biontex K2® Transfection System".

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MATERIALS AND METHODS

Cell culture and transfection

1- 150,000/well BxPC-3 cells were seeded in 12 well plates in 1 ml RPMI-1640 supplemented with 10% fetal calf serum, and 5 mg/ml gentamicin.
2- Cells were maintained in a humidified incubator at 37°C and 5% CO_2 until they reached a confluence of 70%.
3- On the day of the transfection, the medium was changed and cells in 528 µl RPMI-1640 complete medium were treated with 12 µl of K2® Multiplier 2 hours before adding the lipoplex. For this, K2® Multiplier was dripped slowly onto the medium and mixed by gently swaying the dishes.
4- For each well of a 12-well dish, the indicated lipoplex solutions were prepared at room temperature:
   - Solution A was prepared by adding 1 µg of plasmid-DNA (EGFP, pcDNA3-null and pcDNA3-MRP4) to 80 µl of Opti-MEM® Reduced Serum Medium in a polypropylene microcentrifuge tube (Eppendorf).
   - Solution B was prepared by adding 2 µl of K2® Transfection Reagent to 78 µl of Opti-MEM® in a polypropylene microcentrifuge tube.
5- Solution A was added to the solution B and mixed by gently by pipetting up and down
6- Transfection solution (approximately 160 µl) was incubated for 20 minutes at room temperature and was applied to the corresponding Wells by slow drop-wise addition to the medium followed by gently swaying the dishes to achieve mixing.
7- Transfected cells were incubated at 37°C and 5% CO_2 for 24 hours, when medium was replaced.
8- To assess the efficiency of Biontex K2® Transfection System two conditions were tested varying the ratio between the K2® Transfection reagent and the DNA:

K2® Transfection reagent	Opti-MEM® for K2® Transfection reagent	DNA	OptiMEM for DNA	Mix Vol final
2 µl	78 µl	0.5 µg (EGFP)	78 µl	160 µl
2 µl	78 µl	1 µg (EGFP)	78 µl	160 µl
2 µl	78 µl	1 µg (pcDNA3.1-null)	72 µl	160 µl
2 µl	78 µl	1 µg (pcDNA3.1-MRP4)	77 µl	160 µl

9- Transfection efficiency was assessed by epi-fluorescence microscopy. The fluorescence density was not quantified, but rather estimated by eye. Protein expression was monitored by immunoblotting for proteins encoded by the transfected plasmids.

RESULTS

Fluorescence microscopy analysis 24 hours after transfection showed successful transfection. Among the examined conditions, using a 1:2 ratio (K2® Transfection reagent/DNA) seemed to be the most efficient with ~80-90% of transfected cells (see image below). Cells show a perfectly healthy morphology, excluding any cytotoxic effects of the transfection system.

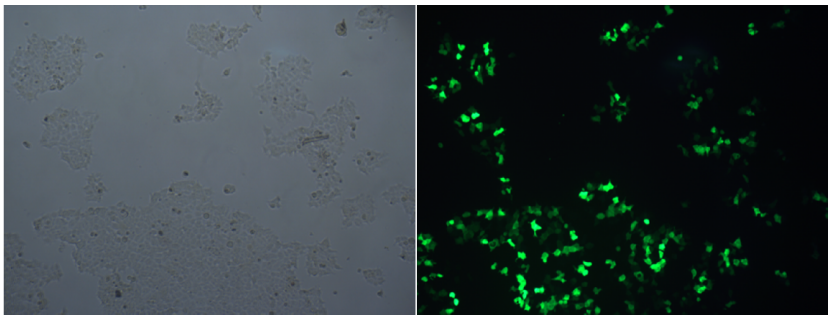

Figure 1: Cell morphology and EGFP expression in transfected BxPC-3 cells. Bright Field (left) and EGFR expression (right).

Plasmid expression was evaluated measuring MRP4 protein levels by immunoblotting. Whole cell extracts from pcDNA3-MRP4 transient transfected BxPC-3 cells showed high levels of MRP4 protein compared to control cells (pcDNA3-null transfected BxPC-3).

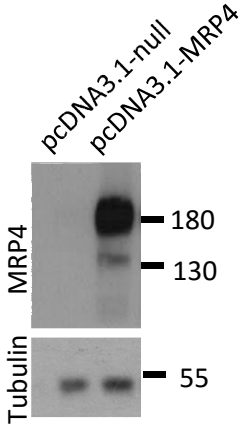

Figure 2: MRP4 expression (~180kDa) was determined by immunoblot in pcDNA3-null and pcDNA3-MRP4 transient transfected BxPC-3 cells.

CONCLUSIONS:

Our results show that the Biontex K2® Transfection System is highly efficient in BxPC-3 cells, even with relatively low amounts of DNA and transfection reagent. Taking into account this cell line is a suitable transfection host, (https://www.atcc.org/Products/All/CRL-1687.aspx), little as 2 µl K2® Transfection reagent and 1 µg DNA per well (in a 12-well format; 700 µl final volume) was sufficient to achieve a ~80-90% transfection rate. Fluorescence microscopy

revealed successful transfection rates without inducing changes in cell morphology or cytotoxicity. By means of Western blot analysis, we can conclude that transfected MRP4 is indeed overexpressed in transient transfected BxPC-3 cells.