K2® Transfection System Technical Note

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Materials :

pCP-Receptor
pEGFP-N1, as control
SMARTpool siRNA receptor
siRNA-OFF-TARGET-siScramble : 5'-3' (Thermo Scientific Dharmacon®)
6-well plate (TPP, Switzerland)
Sterile microtubes (Eppendorf)
Trypsin-EDTA (Biochrome, Germany)
DMEM + 20% fetal calf serum (Invitrogen)
OptiMEM (Invitrogen)
MCF-7 breast cancer cells (cultured according to ATCC, without antibiotics)

Transfection reagent :

K2® Transfection System

Transfection of siRNA BMPR2 procedure:

3 x 10^5 MCF-7 cells/well were seeded into a 6-well plate (2 ml medium/well) and incubated for 24 h.
On the following day, transfection was performed with K2 as follows:
Either 2.5 µg DNA or 75 pmol of siRNA were transfected per well.
Two 1.5 mL microtubes were prepared for each DNA or siRNA transfection. Per tube 130 µL OptiMEM were provided and 6.4 µL of K2 were added, continued with gently mixing. In the other tube DNA or siRNA was added, and mixed gently.
K2 and DNA/siRNA were blended by gentle pipetting, followed by 20 min of incubation.
The mixture was added to cells by distributing single drops in the well.
The cells were then incubated for 4 h, followed by medium change in order to minimize toxic effects of the transfection reagent.
24h after transfection, cells were splitted into 6 wells of a 6 well-plate. 96h after transfection, cell were lysed and total RNA and cDNA were prepared.
Analysis of RNA was performed by quantitative RT-PCR as described in Kubisch et al.(1)

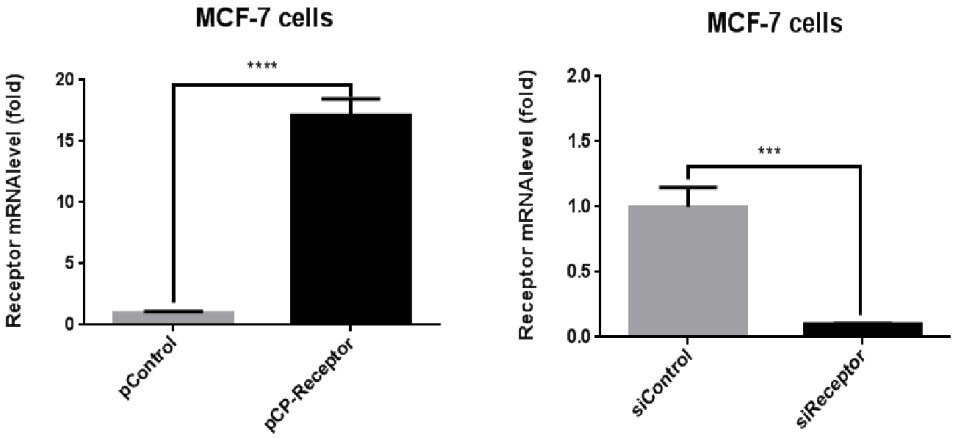
Calculation for DNA transfection

	Opti-MEM	K2	DNA
tube 1	130	6.4 µL	-
tube 2	130	-	2.5 µg

Calculation for siRNA transfection

	Opti-MEM	K2	siRNA
tube 1	130	6.4 µL	-
tube 2	130	-	75 pMol

Results:



Conclusion :

pCP-Receptor and siRNA receptor were successfully transfected in the same conditions (cell line / confluency / amount of reagent) without further optimization, showing great efficiency and limited toxic effects, making K2 an effective and easy to use transfection reagent.

Reference :

(1) Kubisch R, Meissner L, Krebs S, Blum H, Günther M, Roidl A, Wagner E, (2013), A Comprehensive Gene Expression Analysis of Resistance Formation upon Metronomic Cyclophosphamide Therapy, Transl Oncol. 2013;6(1):1-9.