# K2® Transfection System Technical Note

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**Materials:**
Plasmids: pTALEN 1/2, peGFP
MCF-7 breast cancer cells (cultured according to ATCC)
6-well plate (TPP, Switzerland)
Sterile microtubes (Eppendorf)
Trypsin-EDTA (Biochrome, Germany)
DMEM + 10% fetal calf serum (Invitrogen)
OptiMEM (Invitrogen)

**Transfection reagent** :
K2® Transfection System (Biontex Laboratories GmbH)

**Transfection of TALEN + peGFP procedure**:
7 x $10^5$ MCF-7 cells/well were seeded into a 6-well plate (2 ml medium/well) and incubated for 48 h, to reach ≥90% confluency.

On the following day, transfection was performed with K2 as follows: Either 3 µg DNA (2 TALEN + peGFP 1:1:1 Ratio) or 1 µg DNA (peGFP) were transfected per well.

Two 1.5 mL microtubes were prepared for each DNA transfection. Per tube 130 µL OptiMEM were provided and 6.4 µL of K2 were added, continued by gently mixing. In the second tube DNA was added and mixed gently. K2 and DNA were blended by gentle pipetting, followed by 15 min of RT incubation.

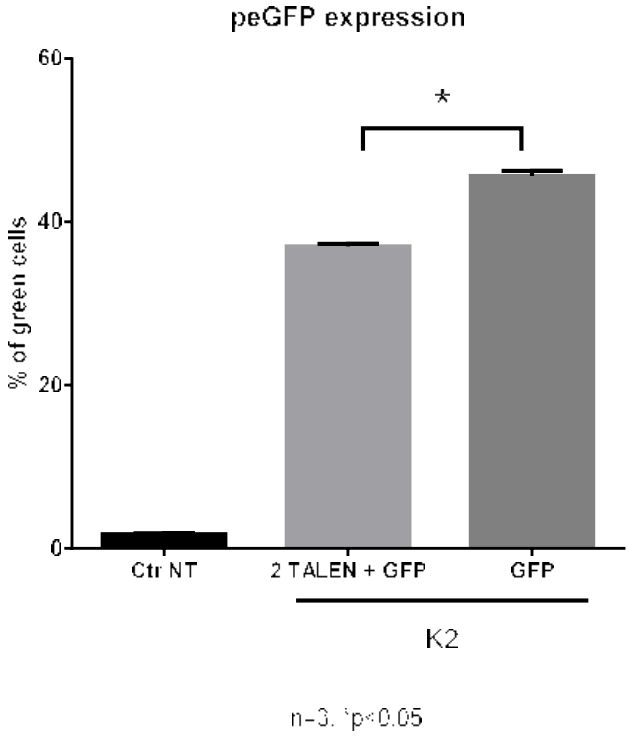
The mixture was added to cells by distributing single drops in the well. The cells were then incubated for 4 h, followed by medium change in order to minimize toxic effects of the transfection reagent.

48h after transfection, cells were trypsinized and prepared to perform FACS analysis to evaluate peGFP expression.

**Calculation for DNA transfection**

| | Opti-MEM | K2 | DNA (2 TALEN + peGFP) | DNA (peGFP) |
|---|---|---|---|---|
| Tube 1 | 130 | 6.4 µL | - | - |
| Tube 2 | 130 | - | 3 µg | - |
| Tube 3 | 130 | 6.4 µL | - | - |
| Tube 4 | 130 | - | - | 1 µg |

**Results:**




**Conclusion:**

New methods in molecular biology, like CRISPR and TALENs, base on the principle of plasmid co-transfection.

For this, we were investigating the efficacy of the reagent K2 by testing mixtures of three plasmids (2 TALEN plasmids + peGFP) vs. transfection of a single plasmid (just peGFP).

The transfections were performed in the same conditions, with the same absolute amount of peGFP in both samples.

Analyzing transfection efficacy via FACS, we observed a statistical difference of just 20% in the number of green cells in the 3 plasmids vs the single plasmid.

The small difference indicates that K2 can be used to transfect single or multiple plasmids with small to none deviation from the standard procotol.