# Overexpression of pcDNA3 containing PEA-15 protein variants plasmids in Cisplatin Resistant Ovarian Cancer Cells EFO27$^{rCDDP2000}$ with K2 Transfection Sytem

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## Materials and Methods

### Cell culture

The cisplatin-resistant ovarian carcinoma cell line EFO27$^{rCDDP2000}$ is from Resistant Cancer Cell Line (RCCL) collection (www.kent.ac.uk/stms/cmp/RCCL/RCCLabout.html). The cells were cultivated as monolayer in medium supplemented with 10% fetal calf serum (FCS), 100 U/mL penicillin and 0.1 mg/mL streptomycin, 2 µg/ml cisplatin (37 °C, 5% CO2). Prior to transfection cells were seeded in 6-well plates with 2 x $10^5$ cells per well in 2 mL without antibiotics and incubated for 24 h. After the transfection, cells were grown in RPMI with 10% FCS and antibiotics and a selection antibiotic G418S to select the transfected cells only. The cells were kept in G418S containing media for an additional two weeks to generate a stably transfected cell lines. After two weeks the cell lysates were prepared and western blot was performed to detect the expression of the PEA-15 protein variants to assess the efficiency of the transfection.

### Transfection of cells

(Please find accurate reagent amounts in the table below)

Cells were treated with 45 µL of K2® Multiplier, 2 hours before plasmid DNA transfection. The multiplier was mixed well with the medium by gently swirling the plates. The plasmids were diluted in IMDM medium without FCS and antibiotics. This diluted DNA was added to the dilution of K2 transfection reagent in IMDM medium, mixed by pipetting once and incubated for 15 minutes. 270 µL of this mixture was added in each well. Again, plates were gently swirled to assure a uniform distribution of transfection reagent. After 24 h the medium was replaced with complete medium containing the selection antibiotic G418S. The cells were kept in the selection medium for two weeks, until all the non-transfected cells are dead. Then the cells are expanded in the selection medium and stocked for further experiments. Efficiency of overexpression was assessed by Western Blot.

| For 1 well | | |
|---|---|---|
| IMDM w/o FCS and antibiotic | 135 | µL |
| K2 Transfection reagent | 10.8 | µL |
| IMDM w/o FCS and antibiotic | 135 | µL |
| Plasmid pcDNA3-HA/ pcDNA3-PEA-15AA/ pcDNA3-PEA-15DD | 2.7 | µg |
| Diluted DNA | 135 | µL |
| Diluted K2 Transfection reagent | 135 | µL |
| Amount Plasmid DNA used per well | 2.7 | µg |

## Results

The expression of PEA-15 containing plasmids was determined by Western Blot for the HA tag. Figure 1 shows the expression of HA, PEA-15AA and PEA-15DD in the cells after transfection with the respective DNA. GAPDH was used as a loading control.

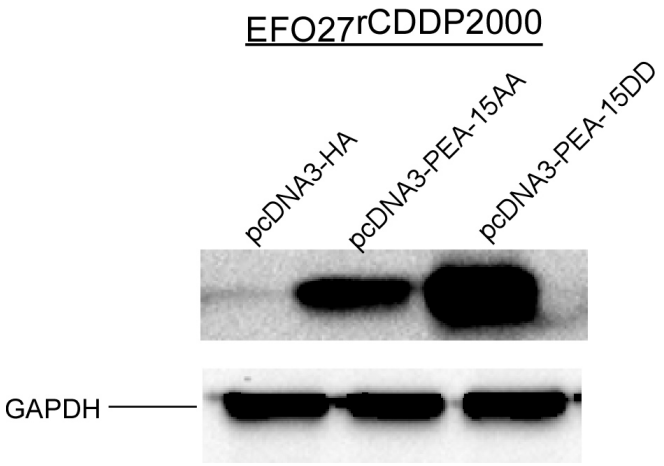


**Figure 1**. Expression of PEA-15 containing plasmids (with two mutations in PEA-15 protein) in EFO27$^{rCDDP2000}$ cells. The HA tag is not visible as it is only 1 kDa.

## Conclusions

The K2 transfection system was successfully applied for transfecting the EFO27r$^{CDDP2000}$ cells with pcDNA3 containing PEA-15 plasmids as shown by the results of the western blot performed for the HA tag.