

## **Overexpression of pcDNA3 containing PEA-15 protein variants plasmids in SKOV-3 ovarian cancer cells with K2 Transfection System**

Shahana Dilruba, Institute of Pharmacy, University of Bonn, An der Immenburg 4, 53121 Bonn, Germany

### **Materials and Methods**

#### **Cell culture**

The SKOV-3 Human Ovarian Cancer Cell Line was bought from ATCC. The cells were cultivated as monolayer in IMDM medium supplemented with 10% fetal calf serum (FCS), 100 U/mL penicillin and 0.1 mg/mL streptomycin (37 °C, 5% CO<sub>2</sub>). Prior to transfection cells were seeded in 6-well plates with  $2.5 \times 10^5$  cells per well in 2 mL without antibiotics and incubated for 24 h. After the transfection, cells were grown in IMDM with 10% FCS and antibiotics for additional two days. Then 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**

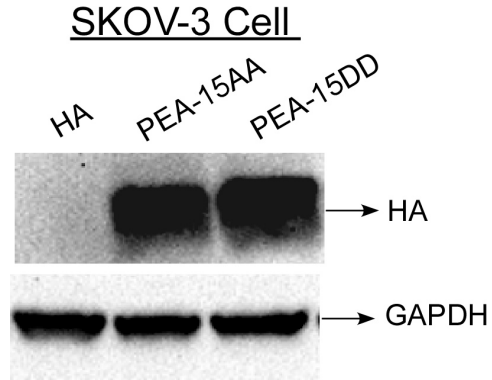
(Please find accurate reagent amounts in the table below)

Cells were treated with 30 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 DNA transfection reagent complex. After 24 h the medium was replaced with complete medium and incubated for additional 48 hrs. Efficiency of overexpression was assessed by Western Blot.

<b>For 1 well</b>	
IMDM w/o FCS and antibiotic	135 $\mu$ L
K2 Transfection reagent	10.8 $\mu$ L
IMDM w/o FCS and antibiotic	135 $\mu$ L
Plasmid pcDNA3-HA/ pcDNA3-PEA-15AA/ pcDNA3-PEA-15DD	2.7 $\mu$ g
Diluted DNA	135 $\mu$ L
Diluted K2 Transfection reagent	135 $\mu$ L
Amount Plasmid DNA used per well	2.7 $\mu$ g

## 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 SKOV-3 cells. The HA tag alone is not visible as it is only 1 kDa.

## Conclusions

The K2 transfection system was successfully applied for transfecting the SKOV-3 cell with pcDNA3 containing PEA-15 plasmids as shown by the results of the western blot performed for the HA tag.