

# Knockdown of PEA-15 gene in SKOV-3 ovarian cancer cells with K2 Transfection System

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#### **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% CO2). Prior to transfection cells were seeded in 6-well plates with 2.5 x  $10^5$  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 to assess the efficiency of the transfection.

#### **Transfection of cells**

On the day of transfection, medium was changed with 1 ml of antibiotic free medium. The PEA-15 siRNA was diluted in IMDM medium without FCS and antibiotics. This diluted RNA was added to the dilution of K2 transfection reagent in IMDM medium, mixed by pipetting once and incubated for 15 minutes at room temperature. 208  $\mu$ L of this mixture was added in each well. Plates were gently swirled to assure a uniform distribution of RNA transfection reagent complex. After 24 h the medium was replaced with 2 ml of complete medium and incubated for additional 48 hrs. Efficiency of siRNA knockdown was assessed by western blot. (Please find accurate reagent amounts in the table below)

For 1 well		
IMDM w/o FCS and antibiotics	100	μL
K2 reagent	2.66	μL
RPMI 1640 w/o FCS and AB	100	μL
siRNA (20 µM)	5	μL
Diluted siRNA	105	μL
Diluted K2	102.66	μL
Amount siRNA used per well	100	pmol
Concentration of siRNA	82.5	nM



### Results

The expression of PEA-15 protein was determined by Western Blot. Figure 1 shows the successful knockdown of PEA-15 gene after transfecting with PEA-15 siRNA using the K2 transfection reagent. Negative knockdown shows no effect on PEA-15 protein expression. HSC-70 was used as a loading control.



**Figure 1**. Successful knockdown of PEA-15 gene in SKOV-3 cells by siRNA using the K2 transfection system (detected by western blot). Transfection of equal amount of negative control siRNA shows no effect on the expression of PEA-15 protein. HSC-70 was used as loading control.

## Conclusions

The K2 transfection system was successfully applied for transfection of PEA-15 siRNA in SKOV-3 cells for knockdown of PEA-15 gene.