**Transfection of SKOV3 human ovarian carcinoma cells using the Biontex K2 transfection system.**

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## Cell culture

SKOV3 cells were cultured in DMEM 10%FBS at 37°C. One day prior to transfection, cells were plated to reach 80% confluency on the day of transfection. 50.000 cells were plated in one well of a 24 well cell culture dish in a total of 500 µl culture medium.

## 1. Transfection of plasmid DNA

Two hours before transfection, either 5 or 10 µl Multiplier solution were added to each well containing 50.000 cells in 500 µl culture medium. Lipoplexes were prepared in serum free medium (Optimem, Life Technologies) at ratios of GFP expression plasmid DNA (pmaxGFP™, Lonza) and K2 transfection reagent of 1:2, 1:4 and 1:6 according to the manufacturer's instructions and complexed DNA was added to each well dropwise after 20 minutes of incubation at room temperature. Medium was changed to 500 µl DMEM 10%FBS after 24 hours and transfection efficiency was determined after 48 hours by FACS analysis (Figure1).

## 2. Results

Best results were obtained using 5 µl of multiplier solution and a DNA: K2 ratio of 1:6 (Fig. 1).

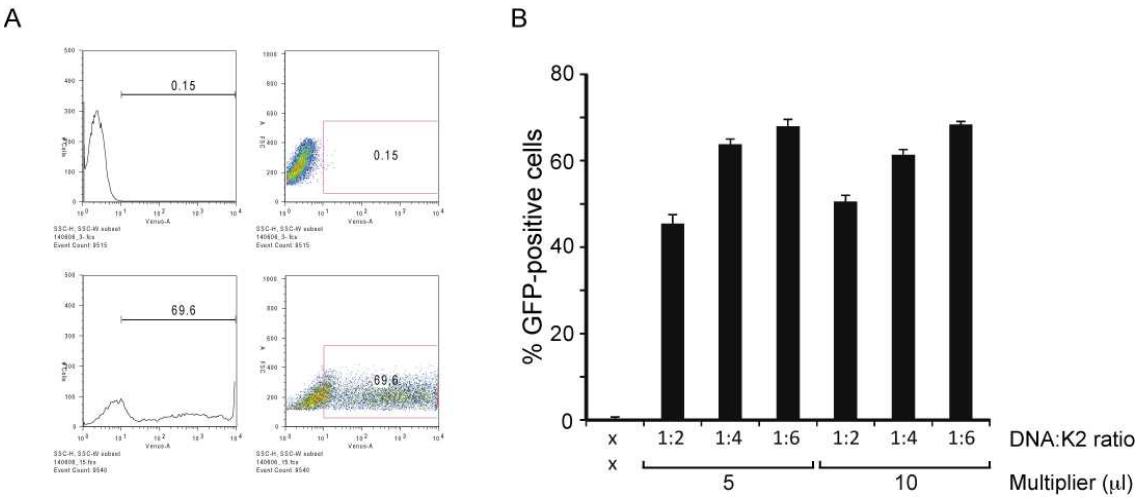


**Fig.1 (A) Analysis of untransfected SKOV3 cells and cells transfected with a 1:6 ratio of DNA: K2 reagent.**

**(B) Quantification of GFP positive cells transfected under varying conditions (x: no plasmid).**

3. Appendix

Table 1: Reagent volumes for 24 well plate format, 1:6 ratio

| Medium | Multiplier | K2 reagent | Optimem | DNA |
|---|---|---|---|---|
| 0.5 ml | 5 µl | 3.6 µl | 30 µl /30 µl | 0.6 µg |