# RNA-transfection of swine kidney cell line (IBRS-2) using "Biontex K2® Transfection System"

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## Materials and Methods

### Cell culture

Swine kidney epithelial cells IBRS-2 were grown in 12-well plates, in Dulbecco´s modified Eagle medium supplemented with 10% FBS, penicillin-streptomycin and L-glutamine. Transfection was performed on subconfluent monolayers (about 90%) split the day before.

### Cell transfection

Cells were treated with K2® multiplier 2 h before RNA transfection. For this, K2 was dripped slowly onto the medium and mixed by gently swaying the dishes. K2® Transfection Reagent was mixed with DMEM (serum-free) and left at room temperature during preparation of the RNA. RNA solution containing different concentrations of 8.000 nt-long viral single-stranded RNA derived from a cDNA clone by in vitro transcription was prepared with DMEM (serum free) and added to the solution containing K2® Transfection Reagent, mixed gently by pipetting up and down, followed by 20 min incubation at room temperature. Transfection solution was applied to cells that were then incubated at $37^0$C and 5% $CO_2$ for 24 h. Then, transfection mixture was removed and replaced with fresh complete medium. Mock-transfection control was performed adding DEPC-water instead of RNA to the mixture. Transfected cells were monitored for development of cytopathic effect (CPE) by optical microscopy as a result of viral growth upon delivery on susceptible cells.

| Surface Area | DMEM | K2 multiplier/cells | RNA/K2 transfection reagent (1:2) |
|---|---|---|---|
| 401 $mm^2$ | 1 ml | 18 µl/0,4x$10^6$ | 500ng/1µl<br>1µg/2µl<br>5µg/10µl<br>10µg/20µl |

**Results**

| RNA amount | CPE detection (time post-transfection) |
|---|---|
| 500ng | - (48 h) |
| 1µg | + (24 h) |
| 5µg | + (24 h) |
| 10µg | + (24 h) |

**Conclusions**

Efficiency: K2® Transfection System showed a similar transfection efficiency on porcine IBRS-2 than other commercial reagents for lipofection commonly used in the lab for long RNA. Only a ≤2-fold decrease in efficiency was observed for K2® Transfection System with the lowest RNA amount (500ng).

Toxicity: no sign of toxic effect could be observed by optical microscopy analysis or any kind of cell alteration in shape or growth capacity in either transfected or mock-transfected cells using K2® Transfection System scaled up to 5µg of RNA and up to 48 h post-transfection. In summary, we found K2 reagent suitable for routine RNA transfection of porcine epithelial cells.