# DNA transfection of human embryonic kidney cells 293T (HEK293T) using "Biontex K2® Transfection System"

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## Materials and Methods

### Cell culture

Human embryonic kidney 293T cells (HEK293T) were cultured in 12 well plate ($0.3x10^6$ cell/well)
in 2.0 ml Dulbecco's Modified Eagle's Medium (Institute of Immunology) containing 10% fetal bovine serum (D-MEM+10%FBS) for 24h at 37°C and 5% $CO_2$. Transfection was performed next day.

### Cell transfection

*(Please find accurate plasmid amounts and experiment conditions in the table below)*
Cells were treated with 20 µl K2® Multiplier per well, 2 hours before DNA transfection. K2® Multiplier was added slowly onto the medium and mixed by gently swaying the dishes.
*Lipoplexes* were prepared in 1,5 ml Eppendorf tube. In tube A, plasmid DNA (pSG5+NPmumps) was mixed with 60 µl D-MEM (Sigma), while in tube B 4,5 µl K2® Transfection reagent was mixed with 60 µl D-MEM (Sigma). DNA solution from tube A was added to the K2 solution in tube B (DNA in K2® Transfection reagent; not the other way around) and mixed by pipetting. Eppendorf tube was left on the room temperature for 20 minutes.
Lipoplex solution was applied on cells slowly, adding drop by drop in a medium, followed by gently swaying the plate to mix lipoplex solution and medium.
Transfections were incubated at 37C and 5% CO2 for 24 hours. Transfection mixture was removed in samples 6 and 7 and replaced with 2 ml of fresh medium D-MEM+10%. In other samples transfection mixture remained to the end of experiment.
Protein expression was estimated 72h after transfection by Western blot for mumps NP protein encoded by the transfected plasmids.

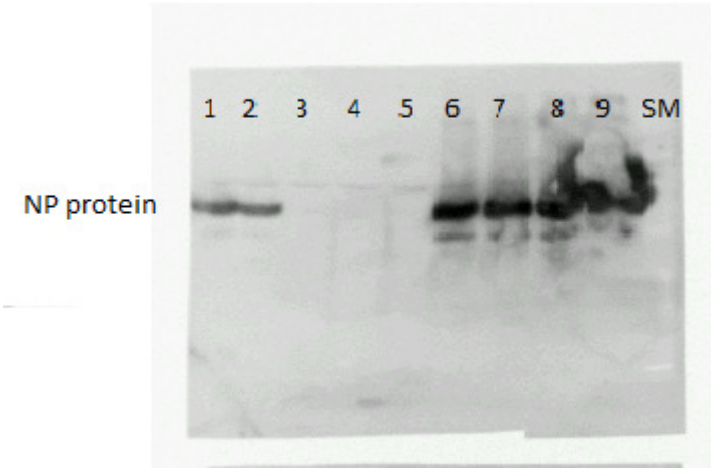
In the same experiment Calcium-Phosphate transfection as a control transfection method was accomplished (Table, Sample 1-2).

### Table

| Sample | DNA Plasmid (µg) | Transfection | Fresh medium |
|---|---|---|---|
| 1-2 | 2.70 | Ca Phosphate | no |
| 3-5 | 0 | Ca Phosphate/K2 | no |
| 6 | 1.35 | K2 | yes |
| 7 | 2.70 | K2 | yes |
| 8 | 1.35 | K2 | no |
| 9 | 2.70 | K2 | no |

## Results

### Western blot




### Conclusions

Plasmid DNA encoding nucleoprotein (NP) of mumps virus was transfected into HEK293T cells using the K2®Transfection System and Ca-Phosphate as a control method. The Western blot shows a band of the expected size (~61.8kDa) in all transfected samples.
K2 transfected cells showed better expression of NP than the Ca-Phosphate transfection cells. Moreover, replacement of cultivation medium 24h after K2 transfection was unnecessary in our experiment.
Cells cultivated 72h in the same medium show healthy morphology and as good expression as the cells in the replaced medium (samples 8,9 vs 6,7).
During the experiment the HEK293T cells showed healthy morphology and no cytotoxic effect was noted.