# Transfection of 293T cells and related cytotoxicity of METAFECTENE PRO

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Human cytomegalovirus (HCMV), one of eight human herpesviruses, represents a major human pathogen causing severe disease in newborns and immuno-compromised patients, i.e. organ transplant recipients and patients with AIDS. Licensed drugs causes a number of serious problems such as nephrotoxicity and have several disadvantages such as low or lack of oral bioavailability. In addition, current treatment has resulted in rising appearance of drug-resistant viral strains, thus new targets with a different mode of action are urgently needed for immunocompromised patients. To date several reports have been deomonstrated that RNA interference (RNAi) might be a new tool for antiviral therapy. The use of vector-mediated shRNA as an antiviral strategy for HCMV has a great potential. Recently we could demonstrate that expression of the HCMV portal (UL104)-specific hairpin shRNAs can silence the target gene (Dittmer and Bogner, 2006). Therefore screening will be an important step to find for the most promising shRNAs. In order to analyse the efficiency of gene silencing of chosen shRNA-sequences plasmids encoding the shRNAs or the target gene will be transiently transfected in 293T cells. To optimize the transfection efficiency we performed analysis with the GFP-expressing plasmid pHM829 by using the novel transfection reagent Metafectene PRO in 293T cells.

## MATERIALS AND METHODS

**Cells and plasmid**. 293T cells were grown in Dulbecco`s minimal essential medium (DMEM) supplemented with 10% fetal calf serum (FCS), 2 mM glutamine and gentamycin (50 µg/ml).
The plasmid pHM829, encoding a ß-Gal-GFP fusion protein (Sorg and Stamminger, 1999), was used for monitoring the transfection efficiency.

**Transfection.** For transient expression of GFP 293T cells (2 x$10^5$ cells/well) were seeded in 24-well microplate. 293T cells at 70% confluence were transfected with Metafectene PRO, a polycationic liposomal tranfection reagent (Biontex Laboratories GmbH, Munich), with different ratios of reagent to plasmid DNA (2:1, 3:1 or 5:1). Cells were washed with DMEM containing 10% FCS prior to addition of 0.5 ml of the same medium. Metafectene PRO were mixed with 30 µl serum-free DMEM while the plasmid DNA was diluted in a volume of 30 µl. Both solutions were thoroughly mixed, followed by incubation for 20 min at RT. The Metafectene PRO-DNA complex was added to the cells and incubated for 16 h at 37°C prior to replacement with serum-containing medium. 48 h after transfection fluorescence analyses were performed. The transfection efficiency was monitored by GFP fluorescence in comparison with DAPI-stained nuclei.

**Cytotoxicity Assay.** Cytotoxicity was measured by using the Cell Proliferating Kit II (XTT; Roche Diagnostics). 293T cells (3 x $10^4$ cells/well) were seeded in a 96-well microplate. At 70% confluency the cells were transfected with various ratios of Metafectene PRO to plasmid DNA. Two days after transfection the cells were incubated with XTT solution for approximately 4 h. The read-out of this assay is the reduction of XTT by viable cells. The resulting formazan dye was quantified with an ELISA plate reader at $A_{492/620}$ whereas the absorbance correlated directly with the cell number.

## RESULTS

Transient transfection was performed by using ratios of Metafectene PRO to the plasmid pHM829 of 2:1, 3:1 or 5:1. In addition, we used two different amounts of plasmid DNA. The highest GFP expression was obtained with 1.5 µl Metafectene to 0.5 µg DNA (3:1; Fig.1).

To determine the cytotoxicity of Metafectene PRO-mediated transfection the Cell Proliferating Kit II (XTT; Roche Diagnostics) was used. Interestingly, while we did not observe cytotoxocity with low amounts of DNA (0.1 µg; Fig.2), amounts of 0.2 µg plasmid DNA resulted in a reduction of viable cells up to 60% (Fig.2).

## CONCLUSIONS

Metafectene PRO results in high efficienct transfection of 293T cells. We recommend to use higher DNA concentration even though the cytotoxicity is increased.


## ACKNOWLEDGEMENT

We thank Thomas Stamminger (Erlangen) for providing the construct pHM829.


## REFERENCES


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**Sorg, G. and Stamminger, T.** 1999. Mapping of nuclear localization signals by simultaneous fusion to green fluorescent protein and to beta-galactosidase. *Biotechnique* **26**, 858-862


## FIGURE LEGENDS

**Figure 1.** Transfection efficiency in 293T cells. The cells were transfected with the indicated ration of Metafectene PRO to the pHM829. Results are expressed as the percentage of GFP fluorescence compared to nucleic DAPI staining. Error bars on the histogram are ± standard deviation from three independent experiments.

**Figure 2.** Cytotoxicity as an effect of Metafectene PRO-mediated transfection by using the Cell Proliferating Kit II (XTT; Roche Diagnostics). Results are expressed as a percentage of viability of a mock transfected control.

Fig. 1

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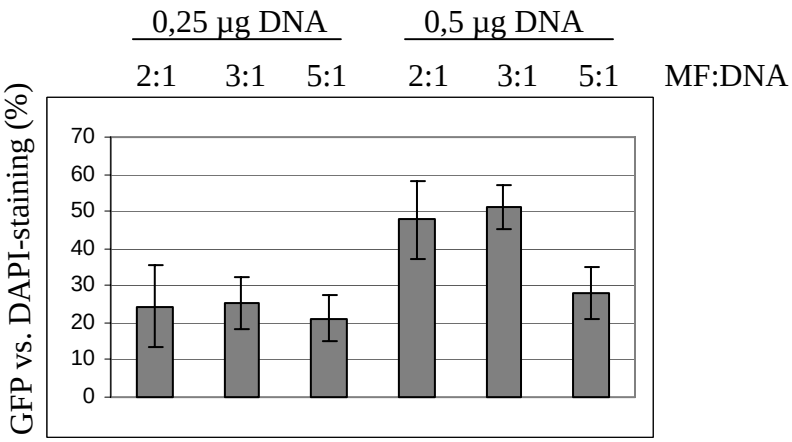

Fig. 2

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