[H S W] HOCHSCHULE WÄDENSWIL		
PROJECT	Introducing pGFP with Metafectene™ or Transfection kit 1 in suspended 293 cells (HEK 293) cultured in protein-free media	
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Mammalian cells, mainly CHO K1 and 293 (HEK 293), were transfected to express recombinant proteins for biopharmaceutical applications. All the transfection methods known – DNA/calcium phosphate co-precipitation, electroporation or lipofection – require defined reaction conditions to obtain high transfection rates. In most cases, the transfection methods are optimised for cells growing in culture media with fetal calf serum (FCS), whereas the actual transfection step has to be performed in serum-free media.

Cells, propagated in medium with or without serum, can be distinguished morphologically: the cells in contact with serum grow adherent, those without FCS in the culture medium are suspended. This phenotypical and biochemical change causes adaptations to establish a universal transfection method for monolayer and suspension cultures.

In transfecting protein-free cultured 293 cells, we tested two different transfection kits and performed the transfection in three different protein-free culture media. The results let assume that the transfection success primarily depended on medium composition.

TRANSFECTION OF 293 CELLS

293 cells (DSMZ ACC 305) were cultured in the following media in a humidified atmosphere at 37°C and a content of 5% CO_2 in 25 and 75 cm^2 culture dishes of Orange Scientific:

1) InVitrus™ (Cell Culture Technologies GmbH) with 10% FCS, 2 g/L Pluronic-F68 and 2 mM L-Glutamine (all from Sigma)

2) InVitrus™ without FCS, 2 g/L Pluronic-F68 und 2 mM L-Glutamine

3) Hektor-G (Cell Culture Technologies GmbH) without FCS, 2 mM L-Glutamine.

We established a method consistent for Metafectene (Biontex) and another buyable lipofection kit (= transfection kit 1), because the assays follow the same transfection principle and were used in the same concentration ratio of DNA/transfection reagent. We performed the following steps:

1) 293 cells were propagated to a cell density of $5 \cdot 10^5$ cells/ml with a viability of 80-100% in 24-well dishes (Falcon).
2) The solutions were prepared in a 96-well plate according to figure1. For one 24-Well 50 µl HEKTOR-G medium were mixed separately with 1.5 µl GFP-plasmid and 3.5 µl transfection reagent, both solutions were put together, and the resulting transfection mixture was incubated during 15-20 min at room temperature in a sterile area.
3) The cells were suspended in the transfection mixture. The transfection cell density was $2 \cdot 10^5$ cells/ml.
4) Incubate for 8 hours.
5) Exchange transfection mixture with fresh culture medium.
6) After 18-72 hours of incubation at 37°C and 5% CO_2, the GFP produced by the transiently transfected cells was controlled microscopically.

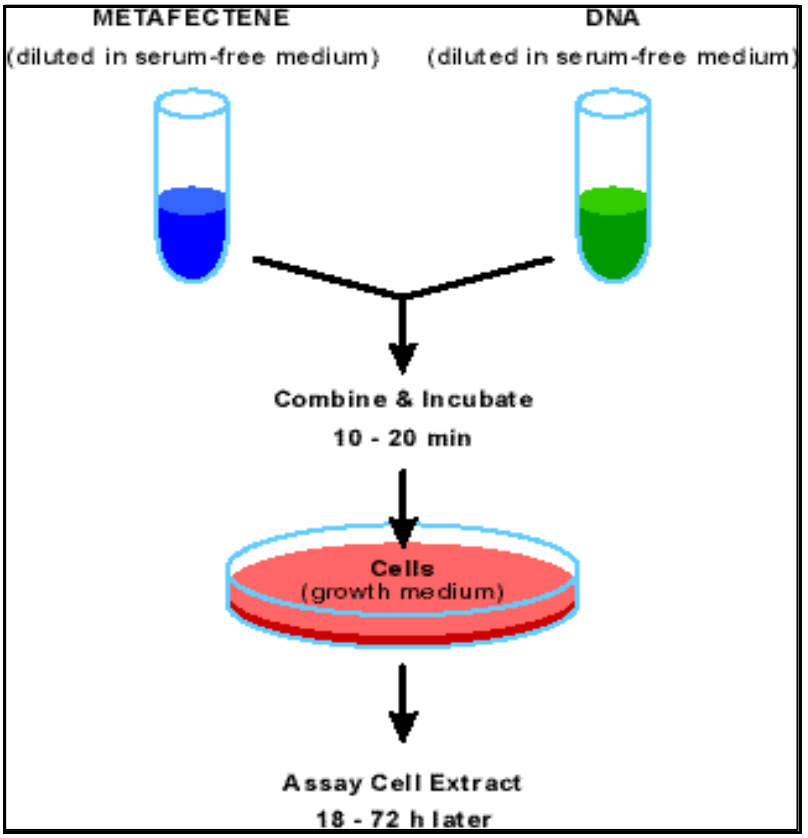
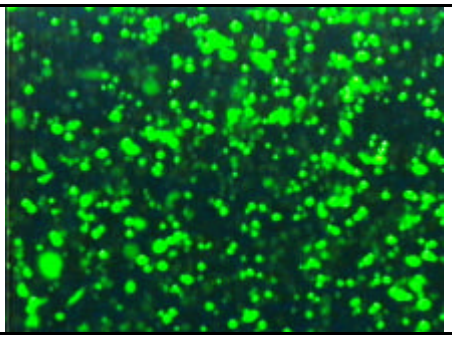
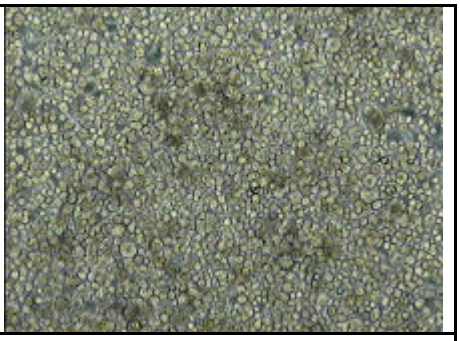
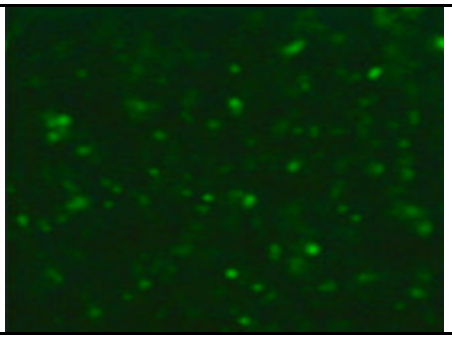
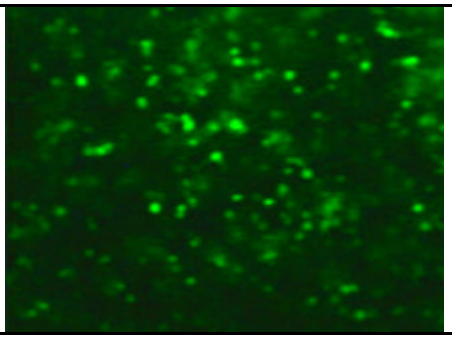
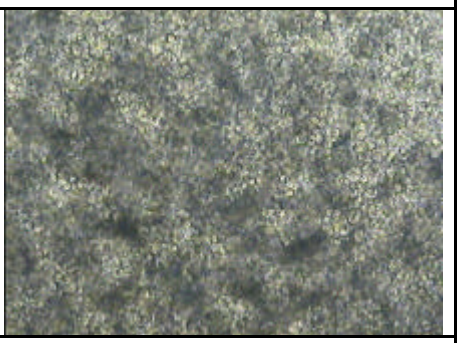


Figure 1: Transfection procedure according to the instruction by the manufacturer.

TRANSFECTION EFFICIENCY

We compared the transfection success of 293 cells: one culture grown in serum-supplemented medium as monolayer, the others in serum-free, protein-free media as suspension. These two appearances of the same cell type have dissimilar transfection rates which was high for monolayers and low for suspensions. To give an impression of the transfection success, we controlled the GFP expression optically with the fluorescence microscope (figure 2).

The transfection rate was highest with Metafectene-Reagent in medium Hektor-G, both in the case of adherent as well as with suspended cells.

HEK-293 InVitrus 10% FCS Metafectene		
HEK-293 InVitrus 0% FCS 0.2% Pluronic F-68 Transfection kit 1		
HEK-293 InVitrus 0% FCS 0.2% Pluronic F-68 Metafectene		

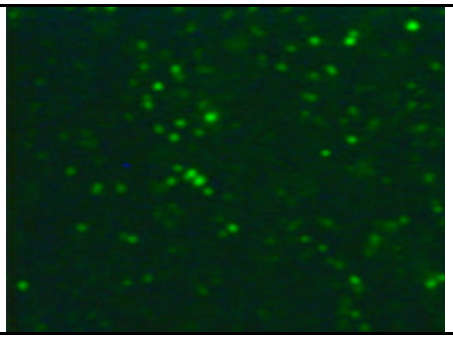
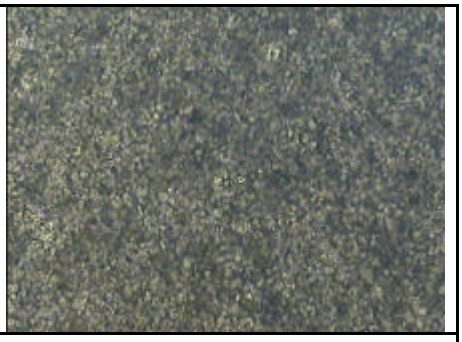
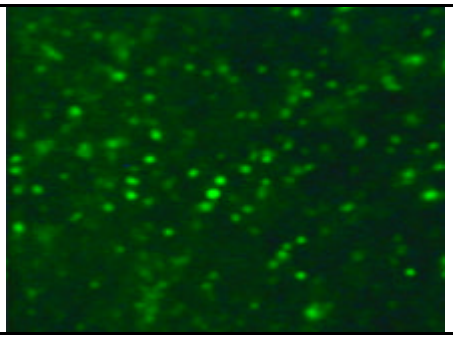
HEK-293 Hektor-G 0% FCS Transfection kit 1		
HEK-293 Hektor 0% FCS Metafectene		no picture (as above)

Figure 2: Transient expression results of HEK-293 with Metafectene and a competitive transfection kit (Transfection kit 1) in the media InVitrus and Hektor-G.