Introducing pGFP with cationic lipids into suspended HEK-293 cells cultured in protein-free media

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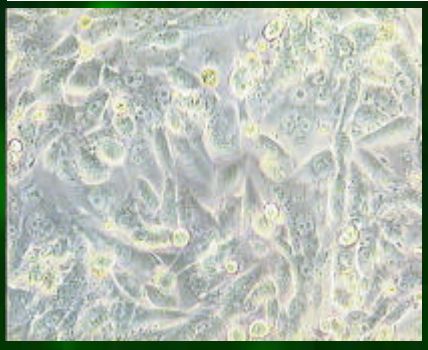
Abstract: HEK-293 were adapted to serum- and protein-free media EX-CELL™ 293 and InVitrus™. The adapted cells underwent morphological changes towards suspension but did not incorporate DNA with the aid of lipofection reagents such as LipofectAMINE 2000™ or Metafectene™. By optimising the transfection procedure using Hektor™ G as transfection medium, we reach higher transfection efficiencies which were comparable to the results obtained with cells in serum containing media in the case of InVitrus™/ Hektor™G, but not with EX-CELL™ 293/ Hektor™G.

Cultivation of HEK-293 in culture media without serum

The 293 or HEK cells (DSMZ No ACC 205) grow as monolayers in InVitrus™ (Cell Culture Technologies GmbH) and 10% fetal calf serum (FCS). The fibroblastoid cells were stepwise adapted to the serumfree medium EX-CELL ™ 293 (JRH) and the chemically-defined, protein-free InVitrus™. The removal of serum prevented the cells to adhere and drove them to suspension. The suspended cells doubled within 24 hours in EX-CELL™ 293 and 17 hours in InVitrus™, compared to 20-24 hours in medium with 10% FCS. In both media, the cells were seeded at a density of $2{\cdot}10^4$ cells ml^{-1} and reached a maximum cell density of $1.3{\cdot}10^6$ cells ml^{-1} in culture flasks with viabilities over 90%.



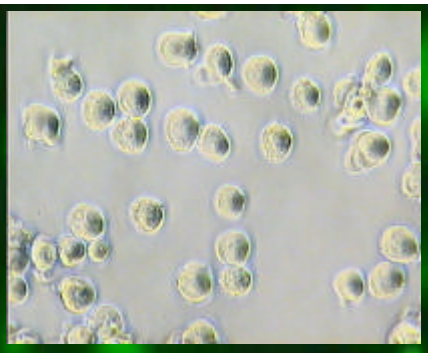


Fig. 1: HEK-293 cells cultured in InVitrus™ and 10% FCS (left) as monolayer and in InVitrus™ without serum as suspension (right).

Table 1: Characteristics of HEK-293 in the media with and without serum in culture flasks.

	InVitrus 10% FCS	EX-CELL 293 0% FCS	InVitrus 0% FCS
Doubling time	20-24 h	24 h	17 h
Maximum cell density	$2.3{\cdot}10^5$ cells $\cdot cm^{-2}$	$1.3{\cdot}10^6$ cells $\cdot ml^{-1}$	$1.3{\cdot}10^6$ cells $\cdot ml^{-1}$
Cell morphology	fibroblastoid adherent	round, suspended, slightly adherent	round, suspended, slightly adherent,

Transfection step in the chemically-defined Hektor G without serum

The ability of a cell to interact with ist environment and to incorporate substances from the medium depends on its surface which can strongly be influenced by changes in medium composition. The loss of serum-components leading to the suspension of the cells necessitates an adjustment of the procedure to transfect mammalian cells with the common lipofection kits established for adherent cells. To better monitor the transfection efficiency, we chose GfP as reporter protein. Transfections of HEK-293 took place with pEGFP-N1(Clontech) in 24-well plates. Preceding transfection, we propagated cells in the following media:

- InVitrus™ and 10% FCS
- EX-CELL™ 293
- InVitrus™.

To study the effects of FCS or hydrolysates (EX-CELL™ 293) in the culture medium on the transfection rate in comparison to the protein-free InVitrus, the lipofections were performed with LipofectAMINE 2000™ (Invitrogen) or Metafectene™ (Biontex) according to the standardized conditions described in figure 2.

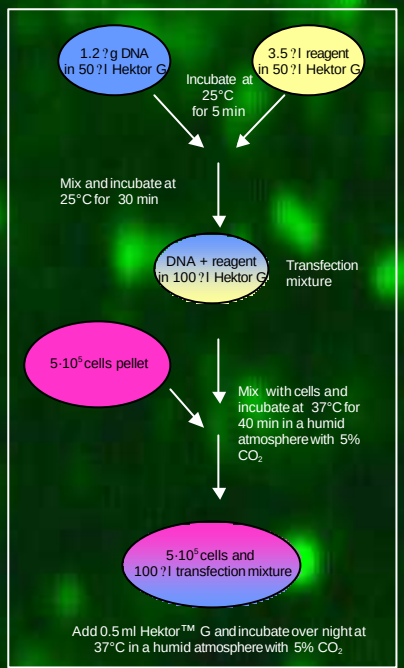


Fig. 2: Conditions to transfect HEK-293 in one well of a 24-well plate with Hektor G.

All cells used for transfection were in exponential growth phase and had viabilities over 90%. All transfections were done in Hektor™ G which was found best suited in preliminary experiments. The DNA and reagent solutions were combined to form complexes within 30 minutes and then added to the cell pellet depleted from propagation medium. The mixture was incubated at ambient temperature for no longer than 60 minutes, then Hektor ™ G was added.
In InVitrus™ with10% FCS we obtained transfection rates of 22% for LipofectAMINE™ 2000 and 29% for Metafectene™. Compared to that, cell cultures in InVitrus™/ Hektor ™ G without FCS gave similar results: 20 and 23% for LipofectAMINE™ 2000 and 25 and 26 % for Metafectene™, respectively. However, when propagated in EX-CELL™ 293/ Hektor ™ G, only 0.1-1% of the HEK-293 were transfected (table2).

Table 2: The HEK-293, grown either in InVitrus ™ with or without FCS or in EX-CELL™ 293, were checked one day after transfection with pEGFP -N1. The transfection rate was given in percent by transfected cells/total cells. We compared the influence of three different propagation media and the lipofection kits LipofectAMINE ™ 2000 or Metafectene™.

Culture medium	Transfection reagent in Hektor™ G	Transfected cells	Total cells	Transfection rate (transfected/total) percent by cells counted
InVitrus ™ 10% FCS	LipofectAMINE ™	503	2252	22
	Metafectene ™	726	2530	29
InVitrus ™ w/o FCS	LipofectAMINE ™	632 562	3213 2454	20 23
	Metafectene ™	903 711	3480 2884	26 25
EX-CELL™ 293	LipofectAMINE ™	27 15	– –	0.5-1
	Metafectene ™	28 26	– –	

Conclusions

Protein-free cultured HEK- 293 were successfully transfected with LipofectAMINE™ 2000 and Metafectene™ . When cultured in InVitrus™, the results were comparable to the cells grown in medium with serum. In the case of EX-CELL™ 293, the transfection rate was very low, this could be due to special medium components impeding the DNA uptake or even to the autofluorescence of the medium, which makes cells with low GfP expression undistinguishable from the background.

Foto: With Metafectene transfected HEK-293, grown in InVitrus