Efficient Lentiviral Vector Production in a Chemically Defined, Blood-Free and Serum-Free Medium, Scalable to the iCELLis® Bioreactor Technology

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HIGH TITER LV PRODUCTION IN A SERUM-FREE MEDIUM

Optimization of parameters for lentivirus production in serum-free medium in flatware

To determine if a serum-free, blood-free, chemically defined medium could be used for lentivirus (LV) production at scale with adherent HEK293T cells, production parameters were first optimized in flatware. Second generation pseudotyped LV was produced in OptiPEAK♦ HEK293t medium, and compared to DMEM + 10% fetal bovine serum (FBS), which is traditionally used for adherent HEK293T.

- FBS inclusion at scale presents numerous problems, including cost, variability, and lack of chemical definition
- OptiPEAK HEK293t is a serum-free chemically defined medium that supports adherent HEK293T cells and is completely free of blood-derived proteins
- We hypothesized that OptiPEAK HEK293t medium could achieve equivalent titer compared to serum-containing media

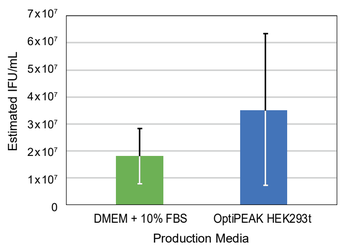


Figure 1
Flatware lentivirus production in a chemically defined, blood-free and serum-free medium

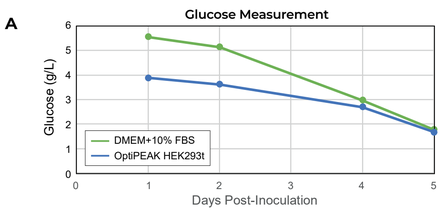
Pseudotyped VSV-g lentivirus vectors were produced in flatware in OptiPEAK HEK293t and DMEM + 10% FBS. HEK293T cells for LV production were seeded at 75,000 cells/cm² 18 hours before transfection. Cells were transfected for 2 hours, followed by a complete media change. LV production in flatware was carried out for 48 hours. After 48 hours, conditioned culture media containing LV virus particles was harvested and titered by functional transduction with HT-1080 cells. Transduced HT-1080 cells were analyzed by flow cytometry 48-72 hours post transduction. N=3 separate experiments, error bars represent standard deviation. Y-axis shows estimated infectious units (IFU) per mL.

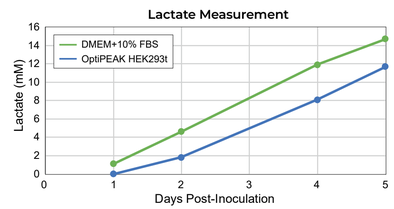
We achieved equivalent or greater functional titer in OptiPEAK HEK293t compared to DMEM + 10% FBS. These results suggest that serum-free media blood-free, chemically defined media has equal to or greater performance compared to FBS-supplemented media, thereby eliminating the problems faced with serum supplementation at scale.

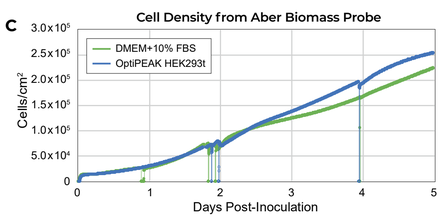
HEK293T GROWTH IN THE iCELLis NANO BIOREACTOR IS HIGHLY SIMILAR BETWEEN OPTIPEAK HEK293t MEDIUM AND DMEM + FBS

HEK293T growth in OptiPEAK HEK293t medium is scalable to the iCELLis bioreactor

- We hypothesized that the same parameters for cell growth and LV production in flatware would be scalable to the iCELLis Nano bioreactor in OptiPEAK HEK293t medium
- The iCELLis Nano bioreactor is a scalable bioreactor technology for adherent cells suitable for commercial production of viral vectors
- 0.53 m² iCELLis Nano bioreactors were inoculated with HEK293T cells at an initial density of 10,000 cells/cm²
- Glucose and lactate were monitored offline by daily sampling
- Growth kinetics were monitored by the Aber Futura♦ biomass probe
- Target cell density for transfection was 70,000 cells/cm² (based on 2D experiments)







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	Pre-Transfection	Post-Transfection
Media linear velocity (cm/s)	2	2
Dissolved oxygen setpoint (%)	95	55
pH setpoint	7.25	6.8

Figure 2 and Table 1
iCELLis Nano bioreactor glucose, lactate, and growth kinetics of HEK293T in OptiPEAK HEK293t (——) and DMEM + 10% FBS (——)

Representative glucose, lactate, and growth kinetics of a production run in OptiPEAK HEK293t and DMEM + 10% FBS in 0.53 m² iCELLis Nano bioreactor. **(A)** Glucose consumption was monitored by daily sampling of the iCELLis Nano bioreactor in OptiPEAK HEK293t and DMEM + 10% FBS. **(B)** Lactate production was monitored by daily sampling of the iCELLis Nano bioreactor in OptiPEAK HEK293t and DMEM + 10% FBS. **(C)** Growth kinetics of HEK293T were monitored in the iCELLis Nano bioreactor in OptiPEAK HEK293t and DMEM + 10% FBS, and the cell density in cells/cm² was calculated from the raw capacitance of the Aber biomass probe[1]. **(D)** Table 1. Optimized set point parameters determined for OptiPEAK HEK293t in the iCELLis Nano bioreactor.

In the iCELLis Nano bioreactor, we achieved equivalent growth kinetics of HEK293T in OptiPEAK HEK293t compared to DMEM + 10% FBS. Glucose and lactate measurement trends were also similar between the two media. However, DMEM + 10% FBS initially started at higher levels for both glucose and lactate. By the end of the production run, glucose levels did not drop below 1 g/L for both media. Lactate, however, did rise above 12 mM in DMEM + 10% FBS. Both media conditions were transfected when the cell density was roughly 70,000 cells/cm² as determined by the Aber biomass probe. These results demonstrate that OptiPEAK HEK293t is scalable to the iCELLis bioreactor and achieves equivalent growth performance as DMEM + 10% FBS.

OPTIPEAK HEK293t MEDIUM PRODUCES HIGH TITER LENTIVIRUS IN THE iCELLis NANO BIOREACTOR

High lentivirus titer was achieved with OptiPEAK HEK293t medium in the iCELLis Nano bioreactor after optimization

- We achieved equivalent growth kinetics in OptiPEAK HEK293t compared to DMEM + 10% FBS, demonstrating that serum-free, blood-free, chemically defined media supports adherent HEK293T at scale
- We hypothesized that we would achieve equivalent functional titer in OptiPEAK HEK293t compared to DMEM + 10% FBS
- We demonstrate that high titer LV production can be scaled from flatware to the iCELLis Nano bioreactor in OptiPEAK HEK293t with overall high viral yield

Four representative LV production runs in 0.53 m² iCELLis Nano bioreactors in either OptiPEAK HEK293t or DMEM + 10% FBS are shown in Figure 3: two pre-optimization runs **(A)** and two runs after optimization of OptiPEAK HEK293t conditions **(B)**. Functional titer was determined by HT-1080 transduction assay. Transduced cells were analyzed by flow cytometry. For each bioreactor run, 900 mL of virus-containing supernatant was collected.

High LV functional titer was achieved in the iCELLis Nano bioreactor in OptiPEAK HEK293t serum-free medium. Optimization of production parameters (transfection cell density and the addition of InVitria Optiferrin♦ transferrin) resulted in an approximately 4X increase in viral titer in OptiPEAK HEK293t compared to DMEM + FBS.

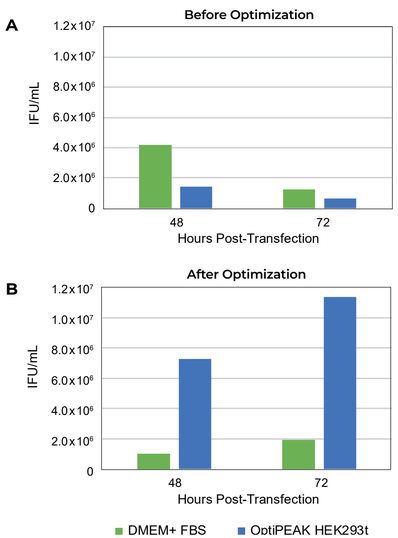

Figure 3
Functional LV titer produced in OptiPEAK HEK293t medium and DMEM + 10% FBS in the iCELLis Nano bioreactor

CONCLUSION

- **In flatware, OptiPEAK HEK293t serum-free medium produces equivalent LV titer as DMEM + FBS**
- **In the iCELLis Nano bioreactor, growth and metabolite profiles are highly similar between HEK293T cells grown in OptiPEAK HEK293t and DMEM + FBS**
- **In the iCELLis Nano bioreactor using InVitria's cell culture protocol, OptiPEAK HEK293t medium produces functional titers approximately four times higher than DMEM + FBS**

Reference

1. Alfano, R. et al. (2020), Implementation of Aber's Futura Biomass Probe in Pall's iCELLis Nano Bioreactor Provides a Robust and Reproducible Method to Assess Cell Density [Poster], ASGCT, < https://www.pall.com/en/biotech/posters-presentations/reproducible-method-assess-cell-density.html>

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