

VIRAL VECTOR PRODUCTION WITH HEK293 CELLS

Simple, reproducible, scalable.

High viral vector and transient protein expression in large scale stirred tank bioreactors with HEK293 cells in a synthetic medium.



One platform for production of viral vectors and transient protein expression – no repetitive media and process development.



BENEFITS

Developed to maximize viral vector replication and transient protein expression with HEK293 cells. The platform consists of several modules which are integrated to each other and finetuned.

One platform for viral vector production and transient protein expression: The same cell bank, same media and same bioreactor settings for viral vector production and transient protein expression. No repetitive media development and process development necessary.

Scalable: Scalability of suspension cells is demonstrated from shake flask to stirred tank bioreactors.

Simple: Ideal from bench to manufacturing scale due the use of standard cultivation methods, e.g. shake flasks in stock culture, stirred tank in bioreactor. The whole process requires minimal human interaction, thus increases process reproducibility.

Highest regulatory quality: No risk for animal derived contaminants in culture media or cell bank do to the lack of animal components.

PRODUCT INFORMATION

The platform is optimized for Poly Ethylene Imine (PEI) mediated gene transfer. This platform is used commonly in 2 applications. First, high viral vector production for gene therapy application. Secondly, for transient expression of recombinant proteins.

This platform is not suitable for active virus infection and for stable protein expression. For this purpose, we have developed another platform.

Cell thaw and stock culture is implemented in shake flask. Cell expansion, vector production and transient protein expression is performed in bioreactors in batch or fed-batch process. The whole process runs in stirred tank bioreactors. Production medium can be combined with feed media and extraordinary high cell concentrations can be obtained up to $3 \times 10^7/\text{mL}$.

HEK293 PLATFORM MODULES

THE HEK293 CELL LINE

The cell line is obtained from Public Health of England and adapted to serum free suspension growth. Cell line is further adapted to resist high shear forces in large scale stirred tank bioreactors. Fully adapted suspension cells are frozen in serum free, animal component free medium. Long term cell bank stability is proven over years in performance testing. Cell bank history and development report are part of cell bank delivery package for high regulatory acceptance.

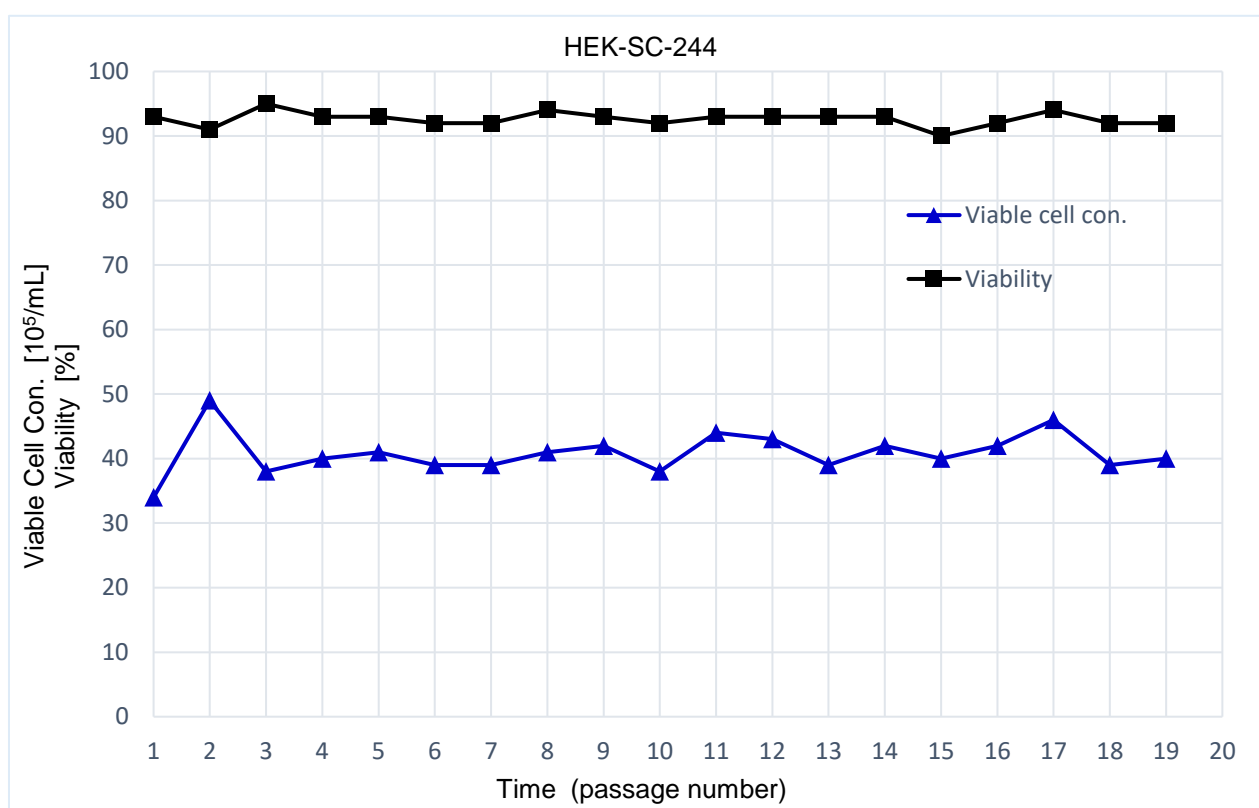


Figure 1: HEK293 suspension cells are cultured in stock culture in shake flasks in IVY medium for 20 passages (60 days). Shake flasks were inoculated with a cell concentration of 5×10^5 /mL and cultures were splitted every third day.



IVY - HEK293 CULTURE MEDIUM AND SUPPLEMENT

IVY medium is developed for stock culture and PEI mediated transfection. ORCHID P medium is a supplement, which can be added after transfection to increase viral vector production and protein expression. Both medium are free of serum, animal components and hydrolysates.

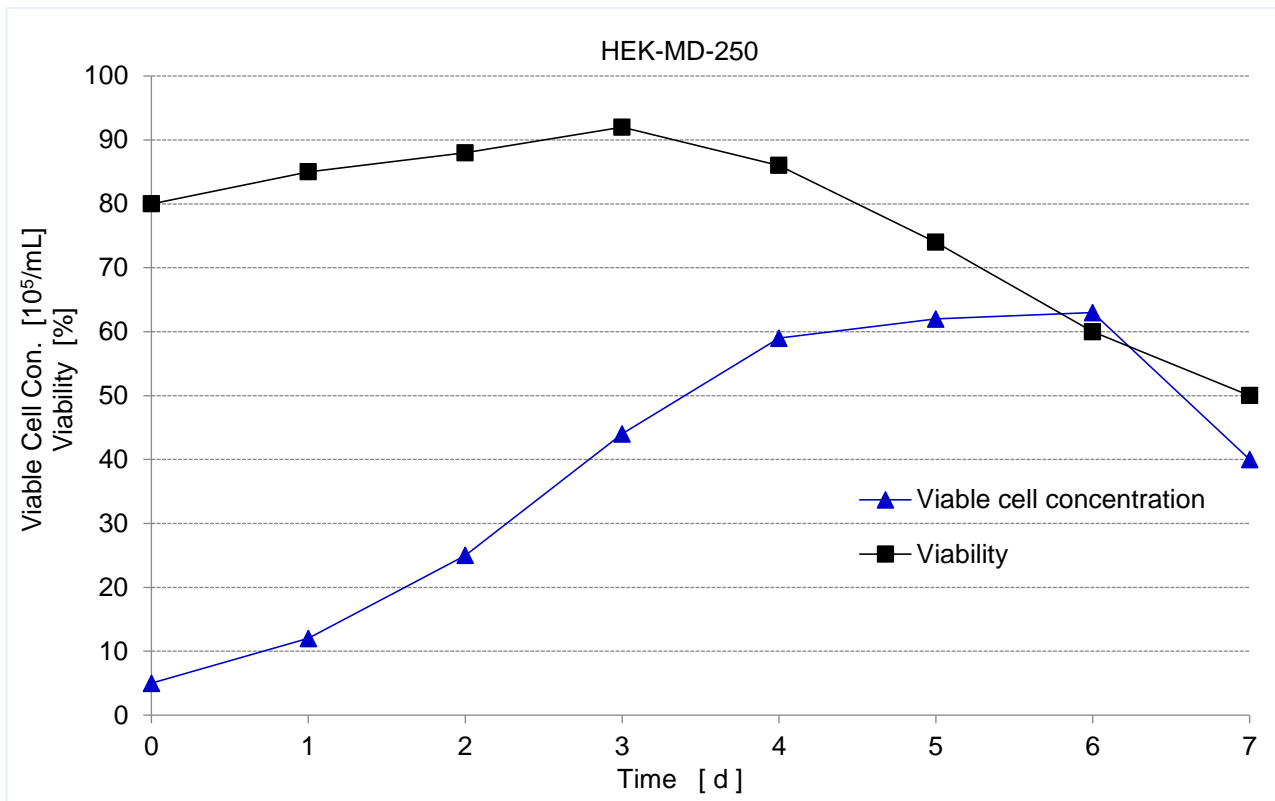


Figure 2: HEK293 suspension cells are cultured in shake flasks in IVY medium in batch. Shake flasks were inoculated with a cell concentration of $5 \times 10^5/\text{mL}$.

HEK293 BIOREACTOR PROCESS

A whole process is developed starting with cell thaw, stock culture, culture expansion and bioreactor steps. The bioreactor process can run in batch or fed-batch modus depending on what is planned to produce. The process is designed for large scale. Detailed process description and support in process transfer are parts of the package.

HEK293 TRANSIENT EXPRESSION

PEI is a popular transfection reagent used at bioreactor scale for transient transfection. IVY medium is specifically developed for PEI mediated transfection. The ionic strength of IVY medium is specifically developed not to interact with PEI and DNA. Thus, high and reproducible transfection efficacy is obtained. This transfection protocol is used in viral vector production and transient protein expression by our clients in manufacturing scale.



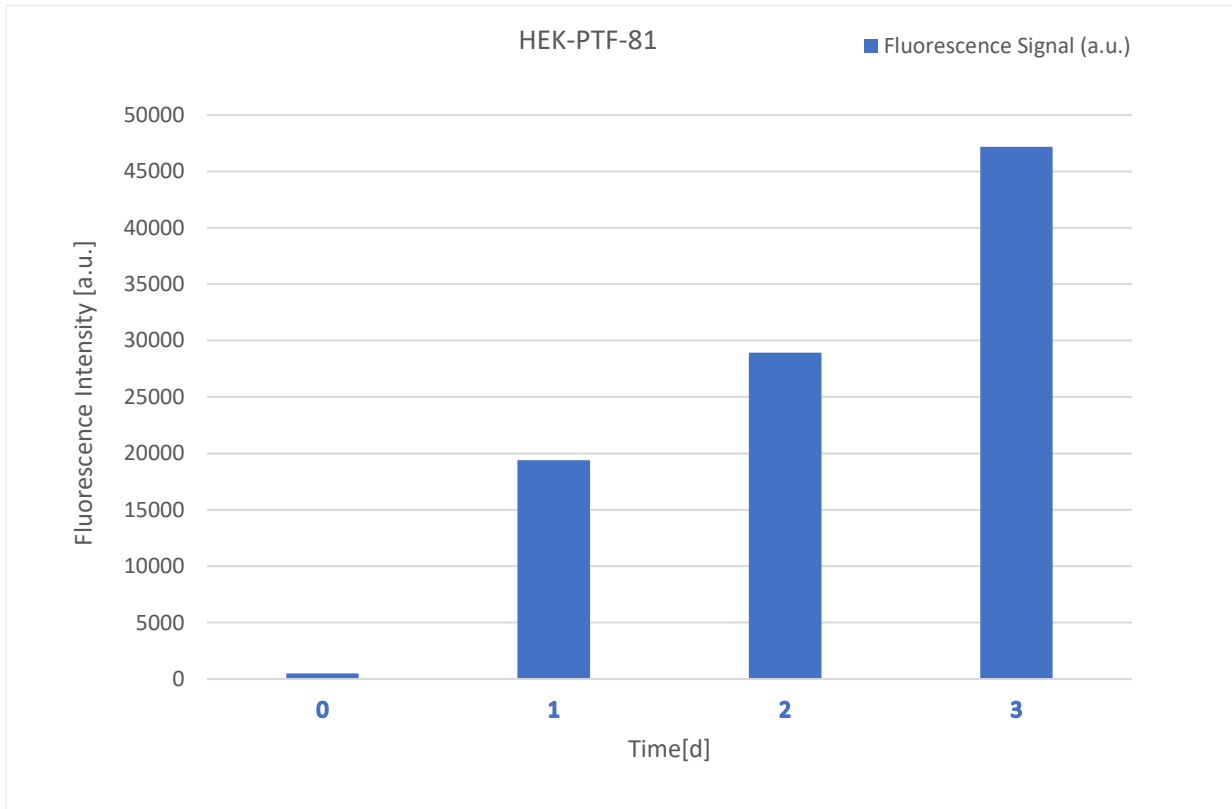


Figure 3: GFP expression in HEK293 suspension cells with IVY medium. Experiment is performed in shake flask. Cells are transfected at day 2 after inoculation. Daily samples were taken and GFP expression was measured. Diagram shows GFP expression after transfection.

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