# PF 293 medium for culture of HEK 293 cells

HYCLONE MEDIA AND SUPPLEMENTS

Transformed HEK 293 cells are used extensively for virus and protein production, research in cell cycle, gene expression, metabolism, receptor binding, and other studies. HEK 293 cells can be cultured as either adherent or suspension cultures. This epithelial cell line was derived from primary human embryonic kidney cells that were transformed using sheared DNA from adenovirus type 5 (1). Transformation allows the cells to be continuously subcultured through a high number of passages, introducing additional beneficial traits that facilitate adenovirus production.

HyClone™ PF 293 was developed and optimized for effective cell growth and performance of human embryonic kidney (HEK 293) cells in a variety of applications. The medium is free of protein and animal-derived components. Here, we compare the performance of PF 293 medium with protein-containing media in HEK 293 cell cultures.

## Establishment of the HEK 293 line

Early research demonstrated the ability of many DNA viruses to transform non-human origin cell lines, but little had been reported on the successful transformation of human cell lines. In 1977, Graham and colleagues at the McMaster University, Hamilton, Canada successfully transformed the primary HEK cell line using a calcium precipitation technique with DNA from adenovirus type 5 (1). The viral DNA was prepared by repeatedly forcing a solution of the nucleic acid through a 22 gauge needle. This process effectively sheared the DNA into small fragments, later to be incorporated by the HEK cells. Two of eight studies, each averaging 20 HEK cultures, produced successfully transformed HEK cells. In these two experiments, after one month of exposure to the viral DNA fragments, a single morphologically transformed colony appeared in one culture.

Isolation of the transformed colonies proved difficult as well. Several attempts at isolation failed. After approximately 75 days, however, a few morphologically transformed cells were observed. These cells were selected by reducing the serum content of the culture from 10% to 2% for several weeks. Once the transformed cells were established, about passage six, the serum content was returned to 10%. Thereafter, Graham refers to a “crisis” phase, which lasted over three months, during which the cells’ growth rate declined substantially. This phase continued until passage 16 when a sharp decrease in population doubling time occurred. This phenomenon also occurred in sublines that had been frozen at passage 6. Following the establishment of the HEK 293 line, cells were maintained in Eagle’s medium supplemented with 10% calf serum and tryptose phosphate broth. Since then, Graham and colleagues have sub-cultured the cells for over 100 passages.

## Use of HEK 293 cells

The process of transformation using the sheared adenovirus type 5 DNA makes the HEK 293 line very sensitive to human adenovirus, and permissive to adenovirus DNA. Adenoviruses, which have traditionally been difficult to cultivate and assay, are more readily cultivated using HEK 293 cells. This high susceptibility is probably due to the fact that HEK 293 cells express one or more adenovirus type 5 specific products that leads to an important use (1). Gene therapy, which exploits the concept that functional biological activities, including gene replacement and repair, can be delivered in the form of nucleic acids, is facilitated by the use of HEK 293 cells for viral vector production.

The HEK 293 cell line has also gained popularity among researchers as viruses have become popular tools for research and genetic engineering. Certain gene therapy applications utilize the infection capabilities of recombinant viruses. Used as vectors for therapeutic genes, these viruses infect and incorporate the genes into the host cell genome. These viruses must be cultivated using susceptible cell lines. Figure 1 shows HEK 293 cells after transfection with recombinant adenovirus carrying the gene for green fluorescing protein (GFP). As shown, this marker gene is expressed within the host cell, and can be observed through fluorescence microscopy.

In addition to using viral vectors, other methods for introducing nucleic acids into a host cell include electroporation, liposomal transfer, phosphate precipitation, and microinjection. Many of these methods are being used with HEK 293 cells for stable or transient gene expression.

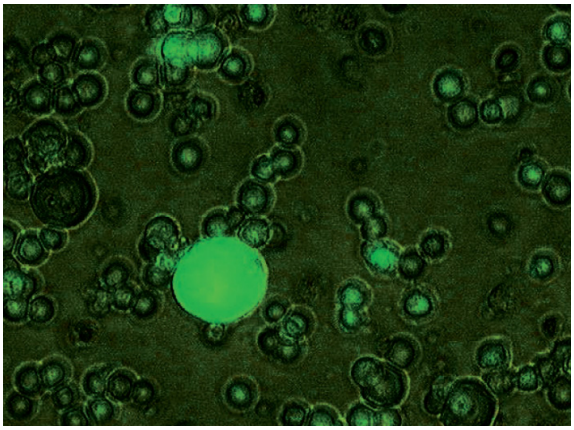

**Fig 1.** Suspension culture of HEK 293 cells in PF 293, following transfection of QBI-AdenoGFP (Quantum Biotechnologies, Inc. Montreal, Quebec, Canada).

## Culture of HEK 293 cells in PF 293 medium

Though HEK 293 cells are adherent in nature (Fig 2), they can be adapted to suspension culture. HEK 293 cells are commonly cultured in T-flasks, shaker flasks, spinner flasks, and bioreactors, in both suspension and microcarrier cultures. Because of the wide variety of uses for these cells, developers of HEK 293 media should consider optimization of the media for various applications such as virus and protein production or cellular transduction. Figure 3 shows growth curves for HEK 293 cells cultured in PF 293 medium as well as in two protein-containing media. Figure 4 shows that simple feeding of batch cultures allows high cell densities to be attained with PF 293.

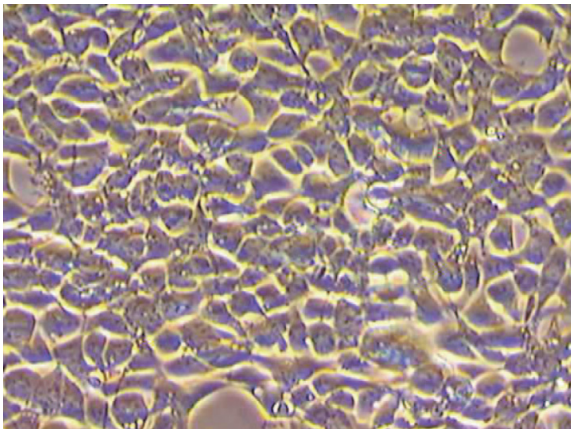

**Fig 2.** Adherent culture of HEK 293 cells.

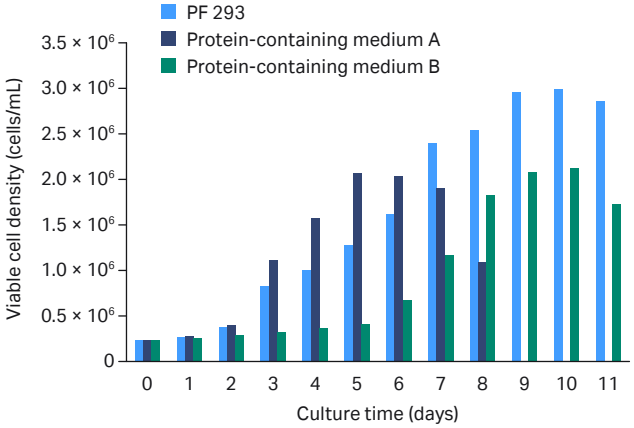


**Fig 3.** Representative growth curves obtained with HEK 293 cells in suspension cultures in 125 mL Erlenmeyer shaker flasks using protein-free PF 293 medium and protein-containing media.

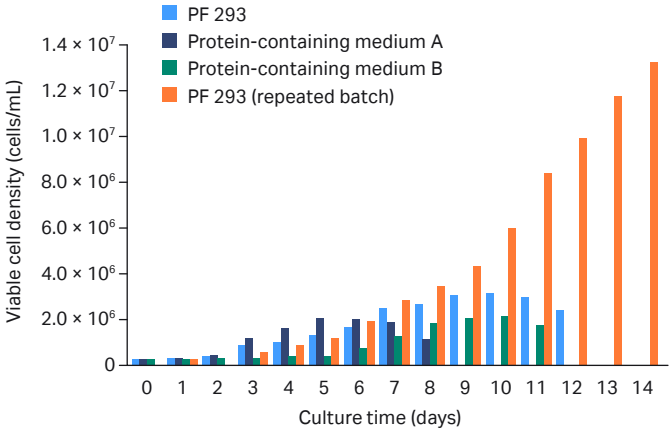


**Fig 4.** Growth curves showing the effect of feeding on HEK 293 cell densities obtained using protein-free PF 293 medium and protein-containing media in standard batch and repeated batch suspension cultures. The repeated batch culture was performed by replacing all PF 293 medium every 24 hours.

## References

1. Graham, F. L., Smiley, J., Russell, W. C., and Nairn, R. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J Gen Virol* **36**, 59–72 (1977).

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