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Application Note

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Adaptation of Mammalian Cell Lines to Serum-Free Suspension Culture

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Abstract

Culturing cell lines in suspension under serum-free conditions offers enhanced flexibility and control over the upstream bioprocess. However, the adapting cells from adherent to suspension cultures is often perceived as a significant challenge.

Our HEK media portfolio is 100% chemically defined and free of serum and animal-derived components, supporting robust and highly productive suspension cell cultures. In this application note, we demonstrate that HEK293 cells can be directly transitioned to both growth and transfection media without compromising cell viability.

Introduction

Traditional mammalian cell culture is usually anchor-dependent and serum-supplemented. Animal-derived components tend to introduce lot-to-lot variability and adventitious pathogens. Cell culture serum also tends to increase immunogenicity and production costs. To overcome these challenges, suspension cultures in non-animal origin (NAO) cell culture media are usually preferred for recombinant protein production or viral vector production due to their suitability to industrial processing and scale-up.

Transitioning from adherent to suspension serum-free culture has its own challenges. A sequential approach is often taken to protect cell viability, a gradual transition requires more process steps and can add significant time and complexity to the adaptation process. Direct transfer to suspension is fast and simple, but cell survival is a concern for some bioprocesses.

In this application note, we demonstrate that direct transfer to suspension (the "do-or-die" approach) is a rapid and effective method to adapt HEK293 cells to serum-free conditions.

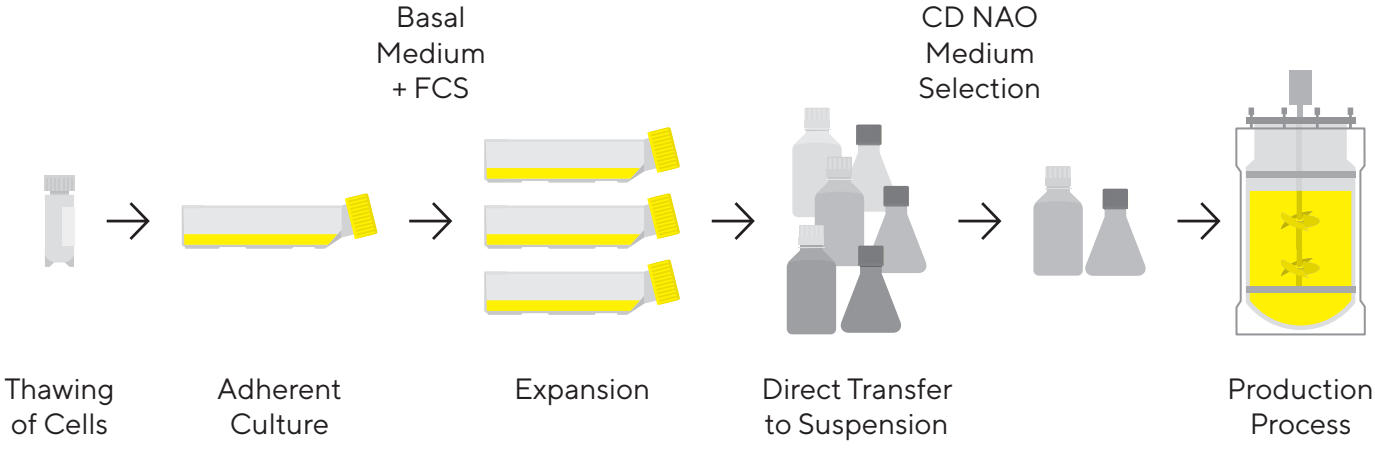
Materials

HEK293 cells were initially grown in adherent cultures using X media. They were then transitioned to growth in either HEK TF Medium or HEK GM Medium, both without foetal calf serum (FCS).

Methods

HEK293 cells were cultured in X medium with X% FBS. To transition to serum-free suspension culture, cells were detached and centrifuged at 200 × g for 5 minutes and resuspended in new, serum-free medium at a concentration of 4 – 6 × 10^5 cells/mL. Cells were maintained at 37 °C and 5% CO_2 at a shaking frequency of 125 – 185 rpm, and passaged cells every 2 – 4 days, depending on cell density. Cells were cultured until viability stabilized at >90%, and growth rates remained constant over 3 – 5 passages.

The Steps Involved in the Direct Cell Line Adaptation Process



Results

Our data indicate that direct adaptation can quickly generate stable cell cultures with high viability.

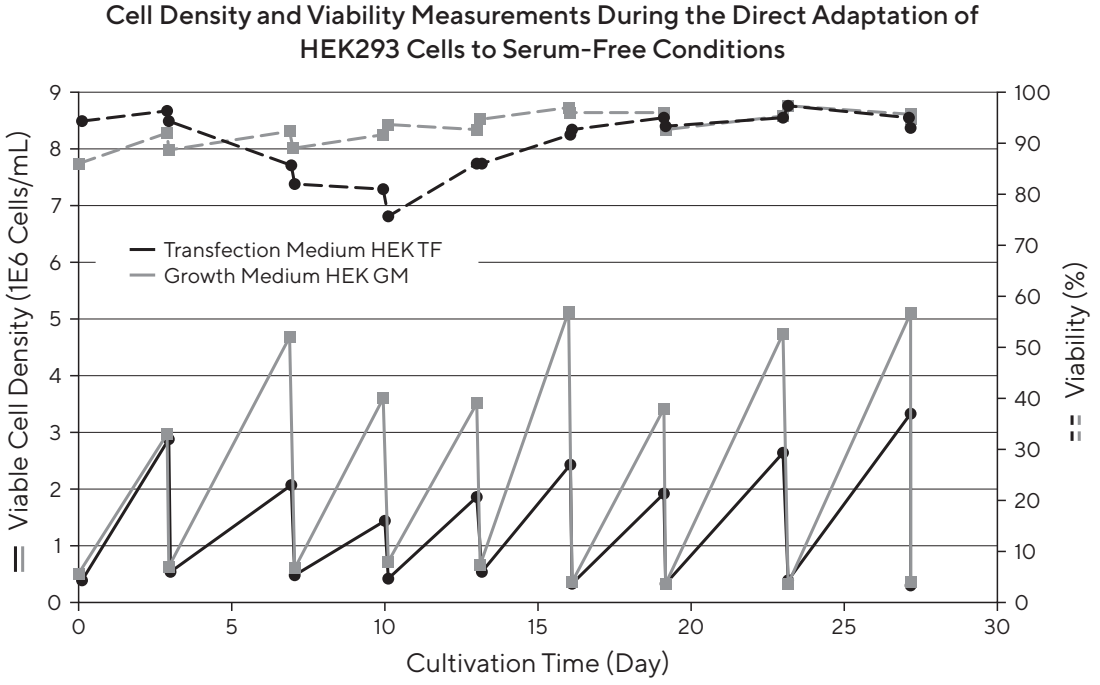


Figure 1: *HEK293 Cell Line B Was Directly Transferred either to Using either Transfection Medium (HEK TF, Black) Or Growth Medium (HEK GM, Gray)*

Discussion

Challenges associated with transition from adherent to serum-free suspension cultivation

This application involves three distinct HEK293 cell adaptations: transition to suspension culture, transition to serum-free conditions, and transition to new media.

Transition to suspension culture

Monolayers are largely limited by the available surface area, while cells in suspension culture can grow in all 3 dimensions, and can reach high cell densities. As a media exchange is not easily performed on suspension cultures, nutrient depletion and toxic metabolite accumulation often become the major limitations in suspension culture. Selecting a suitable, chemically defined culture medium that is proven to support high cell densities, like the HEK ViP media family, is an important first step.

Cells that grow in suspension, either in a shake flask or bioreactor, are exposed to shear stress. Therefore, the culture medium should contain a suitable surface active and protective agent (surfactant). Sartorius' cell culture media contain suitable surfactants that protect cells from shear stress while enabling an efficient production yield.

In addition, shaking speed or stir speed should be chosen carefully to minimize shear stress. Since suspension cultures can support higher cell densities, you should use a richer medium that supports high cell densities and high product yields. Cells can be adapted to the serum-free (e. g., surfactant-containing) version of the previously used basal cell culture medium, followed by transitioning to a richer medium. Alternatively, cells can be directly adapted to the final medium (preferred approach).

Transition to serum-free conditions and new media

Lack of serum exposes the cells to a range of stress like missing growth factors and other serum proteins. Albumin, for example, is an abundant serum protein that can bind and sequester a wide range of ligands, including ions and small molecules. Its absence can change the availability or dose-response relationship of certain cell culture media components. This also has an implication on the use of antibiotics. It is well-known that antibiotics' activity is higher in serum-free media than those containing serum. If antibiotics can't be avoided, their concentration should be reduced. Any additional stress during the adaptation, for example, induced by pH and temperature shifts, should be avoided.

Conclusion

When adapting cells to suspension and serum-free culture, you can either follow the direct approach described in this application note or the more time-consuming, sequential process. Our results indicate that the direct approach enables rapid and robust transition of HEK293 cells from adherent to suspension culture.

We recommend carrying out direct adaptation and sequential adaptation procedures in parallel. After 2 – 3 passages, it will become apparent whether the direct approach has been successful in your process or whether alternative, gentler methods have to be explored.

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