Taking a leaf out of the mAb manufacturing book: media panels for AAV production

Keywords
HEK293, adeno-associated virus (AAV), cell culture media, media panels

Addressing the process development challenges currently being faced by gene therapy manufacturers is critical to successfully progressing gene therapies to the clinic. Currently, the industry is encountering significant challenges when it comes to obtaining suitable titers for commercial scales. Despite being the vector of choice for many gene therapies presently in development, HEK-based adeno-associated virus (AAV) manufacturing processes are especially difficult to optimize because of the complexity of the vectors and the number of factors that can influence their production. This, combined with the accelerated pathways implemented by regulatory bodies for these life-saving therapies, means effective and rapid process development is particularly important. Cell culture media optimization is a key step during process development for producing high titers and ideal product quality. Medium composition has a direct impact on cell growth and transfection efficiency, which means that using the optimal formulation is crucial to achieving a successful manufacturing process and meeting challenging commercialization timelines.

Classic media optimization processes

Traditionally, there are three potential approaches manufacturers use to optimize their medium. These are catalog, customized catalog, and in-house formulations. Catalog formulations are available off-the-shelf from suppliers, where the burden of formulation development lies with the supplier. These formulations are typically a favorite of AAV manufacturers, as they are the fastest solutions to integrate during process development. However, these catalog formulations may not be optimized for a specific cell line, AAV serotype or pseudotype, or transfection method.

gibco

As such, many manufacturers will opt to customize these catalog formulations, modifying the composition to improve results. While this approach may not yield perfect results, it is significantly faster than developing an in-house medium.

In theory, the development of an in-house medium specifically to improve titers and productivity is the ideal solution. Unfortunately, the main drawbacks of this approach are that it could take up to 1–2 years to optimize it, compared with 1–3 months to customize an existing catalog medium, and it is significantly more expensive.

All these options, however, have the same main challenge: finding the optimal starting point. With limited information on the key drivers of productivity in AAV processes, it can be difficult to determine which components to alter. This is especially challenging because of the lack of standardization across the industry.

The media panel revolution

Media suppliers first addressed the challenge of optimizing media to help manufacturers improve mAb production using CHO cells. The solution? Media panels.

Panels, consisting of several nutritionally diverse, chemically defined, animal origin–free formulations, were designed to give manufacturers a starting point to accelerate their media optimization for their specific CHO cell clone. The limited public formulation information that is typically available for catalog media makes it challenging to set up truly diverse media evaluations. The diversity in conjunction with a collaborative supplier, available with a well-designed media panel, makes it simpler to identify key components that are driving productivity and quality. By identifying these key drivers earlier in the process, development times and costs can be reduced. Depending on in-house capabilities, panels can be used as individual formulations or can be combined with supplements to further optimize the process.

Central to maximizing the utility of media panels is leveraging the expertise of the supplier's field application scientists (FAS) and dedicated R&D team. These specialist teams can provide support with the identification of a path forward once the panel formulations have been evaluated, which may include additional analysis and optimization. By reviewing growth, titer, and product quality, it is possible to use techniques such as spent media analysis to make well-informed decisions about further optimization, or even implement nutrient add-back strategies to boost productivity.

This method of process optimization is well established among mAb manufacturers and has transformed process development, helping manufacturers achieve as much as a 70% increase in titers [1]. The chemically defined formulations available as part of panels reduce batch-to-batch process variability, helping enhance consistency and productivity. By leveraging media panels, mAb manufacturers have made significant improvements in the efficiency of media optimization and have increased the speed-to-market of their mAbs.

Applying these methods to HEK293 processes to facilitate the manufacture of AAV-based gene therapies, in particular, has the potential to drive significant advancement of the industry.

Translating success to HEK cells

With the gene therapy industry still very much in its infancy, lessons learned from CHO manufacturing processes could be instrumental as the industry progresses toward the commercial manufacture of these new modalities. Applying these methods to HEK293 processes to facilitate the manufacture of AAV-based gene therapies, in particular, has the potential to drive significant advancement of the industry.

Based on key experience gained during the development of mAb-based media panels, the AAV manufacturing industry now has access to a media panel to accelerate their media optimization processes. The Gibco™ Viral Vector HEK Media Panel addresses the challenges facing the industry, applying methods that have been highly successful in mAb manufacturing [2]. The panel contains five diverse formulations specifically designed for AAV production using HEK293 cells. Along with the panel, FAS consultation and access to R&D team experience is provided as support in the identification and optimization of the ideal medium. By using the panel, AAV manufacturers can now enjoy improved efficiency, an increase in speed-to-market and, crucially, help to meet challenging regulatory deadlines.

It is also key to consider the future of the process when undertaking media selection and optimization. The rapid acceleration of gene therapies means a GMP-compliant workflow should be established as soon as possible, meaning a scalable solution is key. Although the formulations used in the panel are not GMP-compliant to reduce cost during the screening process, they are available at larger scale in different formats and manufactured at a GMP-compliant facility. This means they can be seamlessly integrated into the commercial process without concerns around variability in performance or supply.

The future of AAV process optimization

Addressing the challenges of process optimization is critical to the commercialization of life-saving gene therapies. Solutions such as media panels being introduced to the industry offer a new opportunity to streamline previously time-consuming processes with specialized formulations designed for HEK-based AAV manufacturing. Combining the panel concept with process development expertise and a reliable supply of media upon scale-up paves the way for the next-generation of advanced therapeutics.

Addressing the challenges of process optimization is critical to the commercialization of life-saving gene therapies.

References

1. Thermo Fisher Scientific (2017) Chemically Defined CHO Media Test Panel Consultation Service. https://assets.thermofisher.com/TFS-Assets/BPD/brochures/gibco-chemically-defined-cho-panel-Brochure.pdf.
2. Martin C, Zatina J (2021) Media panels for AAV manufacturing: Finding the formulation for success. *Cell Culture Dish*. https://cellculturedish.com/media-panels-for-aav-manufacturing-finding-the-formulation-for-success/.

Learn more at **thermofisher.com/gibco-gene-therapy**