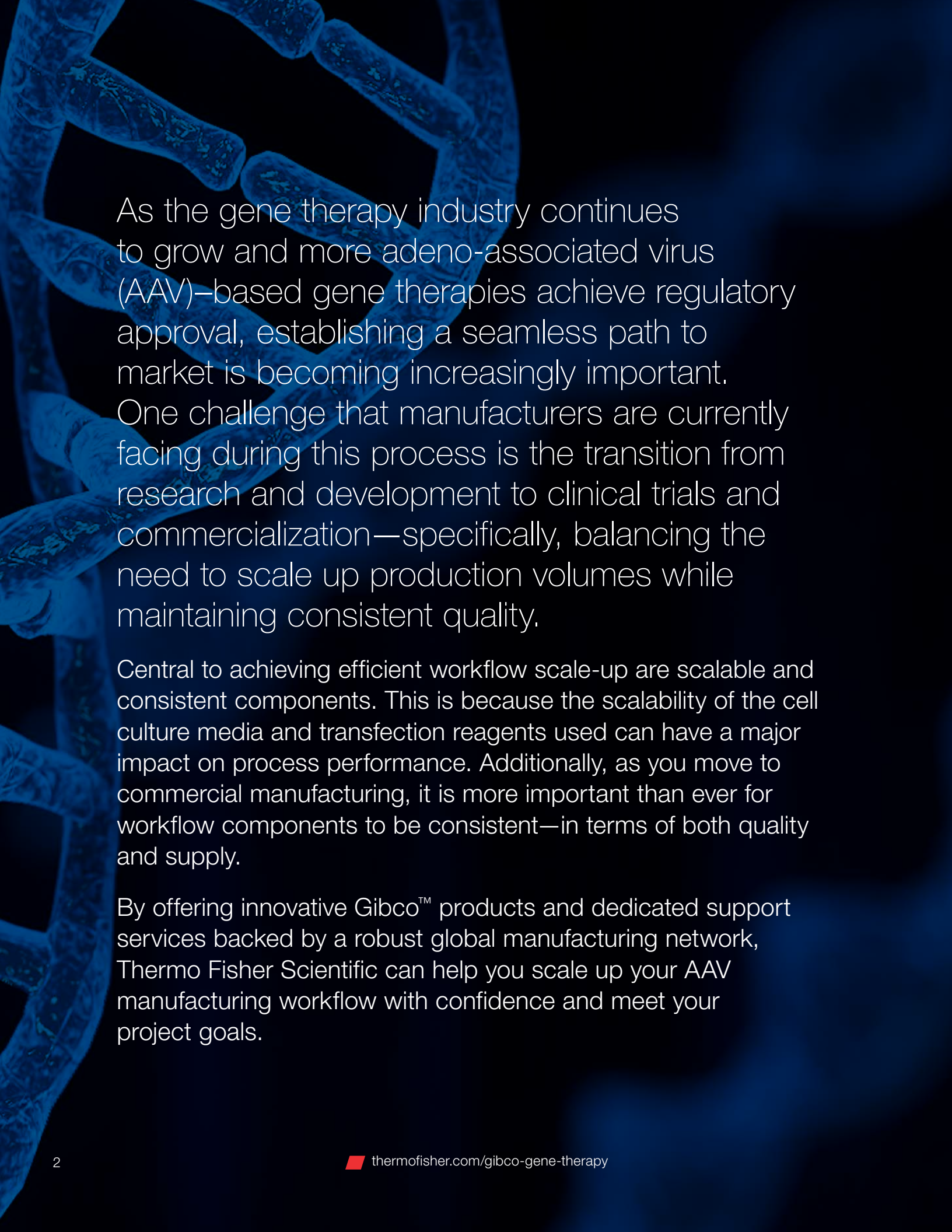




AAV manufacturing

Scaling up AAV production with Thermo Fisher Scientific gene therapy solutions



As the gene therapy industry continues to grow and more adeno-associated virus (AAV)–based gene therapies achieve regulatory approval, establishing a seamless path to market is becoming increasingly important. One challenge that manufacturers are currently facing during this process is the transition from research and development to clinical trials and commercialization—specifically, balancing the need to scale up production volumes while maintaining consistent quality.

Central to achieving efficient workflow scale-up are scalable and consistent components. This is because the scalability of the cell culture media and transfection reagents used can have a major impact on process performance. Additionally, as you move to commercial manufacturing, it is more important than ever for workflow components to be consistent—in terms of both quality and supply.

By offering innovative Gibco™ products and dedicated support services backed by a robust global manufacturing network, Thermo Fisher Scientific can help you scale up your AAV manufacturing workflow with confidence and meet your project goals.

Contents



Plasmid development and manufacture 4



Cell expansion 5



Transfection 6



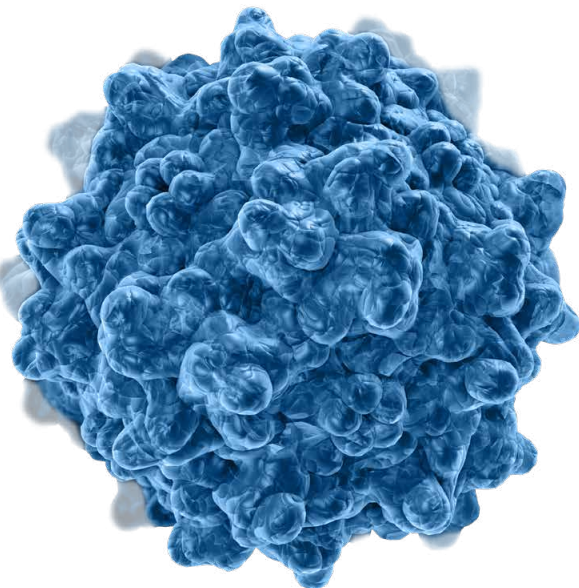
Viral vector production and optimization 7



Supply and quality assurance 8



Custom gene therapy solutions 9





Plasmid development and manufacture

No matter which cell line or transfection method is being used, the first step in most AAV manufacturing workflows is the production of recombinant DNA plasmids. Due to its low cost and relatively fast growth rate, *Escherichia coli* (*E. coli*) is the most popular production system for plasmids and is typically cultured in high-density fed-batch suspension cultures.

Given their central role in AAV production, maximizing plasmid purity and quality has become an increasingly important consideration for manufacturers. In particular, it has become clear that using plasmid DNA that is of the highest purity and free of any contaminants—such as phenol, sodium chloride, and endotoxins—or animal-origin material is vital for optimizing transfection efficiency. Critically, these stringent requirements can only be met using high-quality and consistent media and reagents.

To help you optimize your plasmid production workflow, we offer a range of products and services for both plasmid development and manufacture. Alongside a broad variety of microbial media products, we can provide high-quality supplements, including plant-based peptones, for *E. coli* culture.



Gibco™ Bacto™ CD Supreme Fermentation Production Medium (FPM)

This product is a chemically defined (CD) and hydrolysate-free fermentation production medium developed to support high-density *E. coli* cultures. By offering a CD alternative to traditional *E. coli* media, Bacto CD Supreme FPM can enable gene therapy manufacturers to improve the consistency of plasmid production and eliminate the risk of bacteriophage and BSE/TSE contamination. The medium also provides full sterilization flexibility, allowing manufacturers to choose either filter sterilization or autoclaving depending on their process requirements.



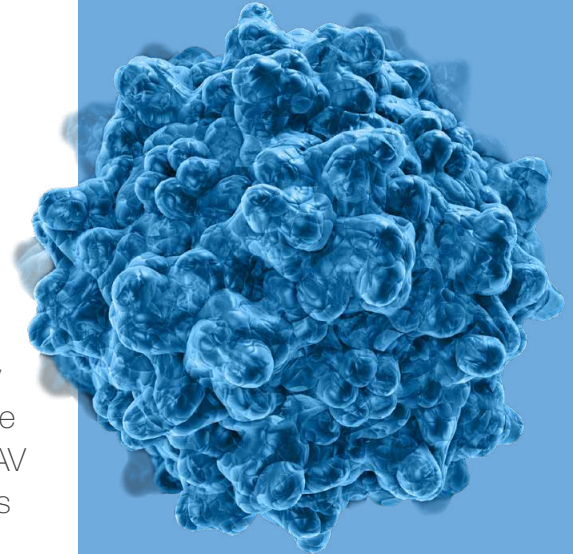
Cell expansion

The next step in the AAV manufacturing workflow is the expansion of a mammalian or insect cell line that is suitable for transfection. Currently, most AAV manufacturers choose to use either HEK293 cells or Sf9 cells for AAV production.

Mammalian HEK293 cells are a popular option for AAV production primarily due to their transfectability and well-known safety profile. Additionally, they are suitable for use in adherent cell cultures, giving manufacturers flexibility depending on their specific process requirements.

Similarly, insect Sf9 cells are also well suited for expansion in high-density suspension cultures, enabling processes to be easily scaled up to commercial volumes. Moreover, the development of commercially available baculovirus expression vector systems (BEVS) has provided a reliable and scalable solution for transducing Sf9 cells and stimulating AAV production.

However, to gain the full benefits of the favorable characteristics of both cell types for AAV production, manufacturers need to choose the optimal cell culture medium for their process. By offering a broad range of high-quality, scalable media solutions, we can help you achieve this and optimize the performance of your process.

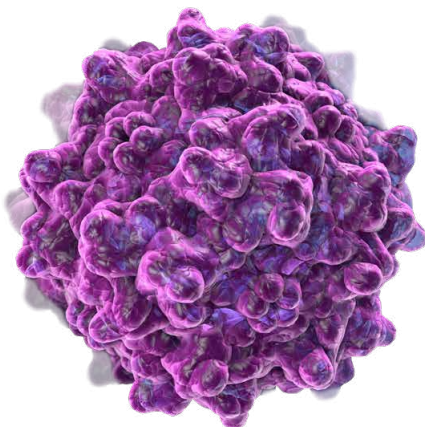


Adherent vs. suspension cell cultures

A key decision that AAV manufacturers need to make when scaling up their process is whether to choose an adherent or suspension culture for cell expansion.

Although adherent processes are often used during R&D, their overall scalability is highly limited, as increased production volumes can only be achieved by increasing surface area. This means significantly expanding facility space, which may not be economically viable. As a result, the use of suspension cultures is typically recommended to enable easy scale up from R&D to commercial manufacture.

Regardless of the production platform used, we offer a broad portfolio of cell culture solutions for both adherent and suspension processes to support manufacturers at all stages of AAV production.



Supporting your transition from R&D to commercial manufacturing

All Gibco™ media intended for bioprocessing have been developed to provide consistent results at different production scales. This means, by choosing Gibco media, you can be confident that the process developed during R&D can be easily scaled to large-scale production without compromising on performance.



Transfection

Following expansion, the cells need to be transfected to enable them to produce AAV. There are a variety of transfection options available depending on the workflow and the cell line being used.

HEK293 transfection

Transient transfection of HEK293 cells for AAV production is usually achieved using a triple plasmid transfection protocol.

There are three main methods for introducing these plasmids into cells:

- Calcium phosphate
- Lipid-based methods
- Polymer-based methods

Depending on the specific process, each method has its benefits and drawbacks, which need to be evaluated before deciding on the approach.

Sf9 transduction

Transient transduction of Sf9 cells is most commonly achieved using a BEVS platform.

These systems typically combine a DNA plasmid with baculoviral DNA to create a recombinant bacmid containing the required genes for AAV production in Sf9 cells. The bacmid can then be transfected into an initial Sf9 cell line to produce recombinant baculoviruses.

These baculoviruses are then harvested and used to transduce a secondary Sf9 cell line, which stimulates the production of AAV.





Viral vector production and optimization

Once the target genetic material has been introduced into cells, they can begin producing AAV. Although this is the final step of the upstream manufacturing workflow, achieving success at this stage is important for overall process performance.

It is vital for AAV manufacturers to maintain their workflow's economic feasibility as it is scaled up. Central to achieving this is maximizing the overall productivity of their process—requiring the optimization of both its performance and efficiency.

Improving AAV titers and quality

The requirements for maximizing process performance are twofold. First, manufacturers need to be able to achieve high AAV titers. They also need to consistently produce high-quality AAV. This is key, as AAV that does not meet the required critical quality attributes will need to be removed during downstream processing, reducing overall yield.

Steps can be taken at all stages of the workflow to help meet both requirements. For example, using a cell culture medium that has been optimized for achieving high titers and consistent quality can

dramatically increase productivity. Similarly, using high-quality transfection reagents that can reliably enable high transfection efficiency can also help you reach maximum process performance.

Optimizing AAV manufacturing efficiency

To attain optimal productivity, AAV manufacturers also need to streamline their workflows. One way you can do this throughout the manufacturing workflow is by using components that have been specifically formulated for ease of use. For example, when using a dry format medium, choosing an advanced format such as Thermo Fisher's proprietary Gibco™ Advanced Granulation Technology (AGT™) medium can have several advantages. In addition to accelerated dissolution times, the AGT format can help speed up media preparation through its osmolality and pH pre-adjustment, enhancing efficiency.



Gibco™ Viral Vector HEK Media Panel

The Viral Vector HEK Media Panel is an innovative solution for accelerating gene therapy development and production. The media panel contains five nutritionally diverse, chemically defined, ready-to-use HEK293 media formulations. It is designed to help AAV manufacturers to identify a formulation that is optimized for high titers, scalable, and can be customized to further increase performance.

Technical support from experienced media development professionals is also provided throughout the evaluation process to help manufacturers expedite their media development and optimization process.



Supply and quality assurance

As with all bioprocessing applications, maintaining consistency—in terms of both quality and supply—of critical process components is vital for a successful AAV manufacturing process. To help you maximize your confidence in this area, we have a robust global manufacturing network, supported by in-depth quality control and raw material sourcing processes.



Manufacturing sites

Our global media manufacturing infrastructure includes facilities in:

- Grand Island, New York
- Miami, Florida
- Paisley, Scotland

This robust network is supported by a detailed equivalency program to enable continuous and uninterrupted global supply.



Quality systems

Across its manufacturing network, Thermo Fisher is committed to achieving optimal product consistency. By operating stringent quality control systems and policies, including a combined total of over 200 facility audits each year, we can deliver dependable gene therapy manufacturing solutions from batch to batch.



Raw material sourcing

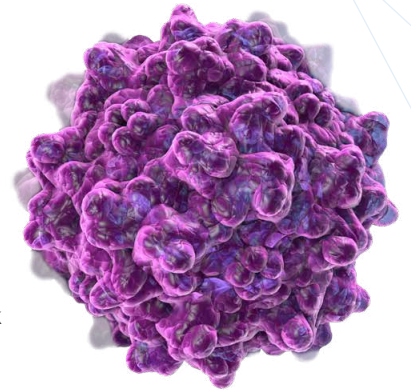
To protect both raw material quality and supply, we have established a rigorous supplier qualification process and a detailed business continuity and risk mitigation plan. Through this, we can offer gene therapy manufacturers around the world a low-risk supply chain with high transparency and minimal variability.



Custom gene therapy solutions

In addition to a broad portfolio of gene therapy solutions, we also offer a range of customization services to meet AAV manufacturers' specific process needs.

Gain access to Gibco™ PD-Express™ Services by choosing to work with Thermo Fisher. These services provide support from teams of experienced professionals who can assess each project's unique requirements and work closely with you to develop solutions that help you meet your goals.



Spent media analysis

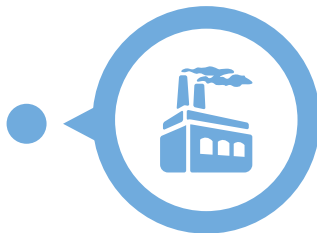
AAV manufacturers can tap into the benefits of our spent media analysis service for rapid quantitative answers about changes to nutrient components and utilization profiles over time. The information gained can help them optimize formulations to meet their process goals.

This service is available for both fresh and spent media analysis,* as well as supplement analysis.* Available assays include amino acids, water-soluble vitamins, and trace elements. Additional assays are available based on your specific needs.

*Biosafety assessment required for all viral vector-containing samples.



**Gibco
PD-Express
Services
for AAV
manufacturers**



Media manufacturing

We offer solutions for media manufacturing at all scales of production.

During process development, manufacturers can utilize the Gibco™ Rapid Prototyping Service, our non-GMP pilot manufacturing facility, to test the manufacturability and scalability of their medium or modify their formulation.

Once they reach commercial scale, they can also leverage its validated cGMP media manufacturing capabilities.



Packaging and format customization

To enable the simple integration of Gibco media into AAV manufacturers' unique workflows, we provide services for custom packaging design.

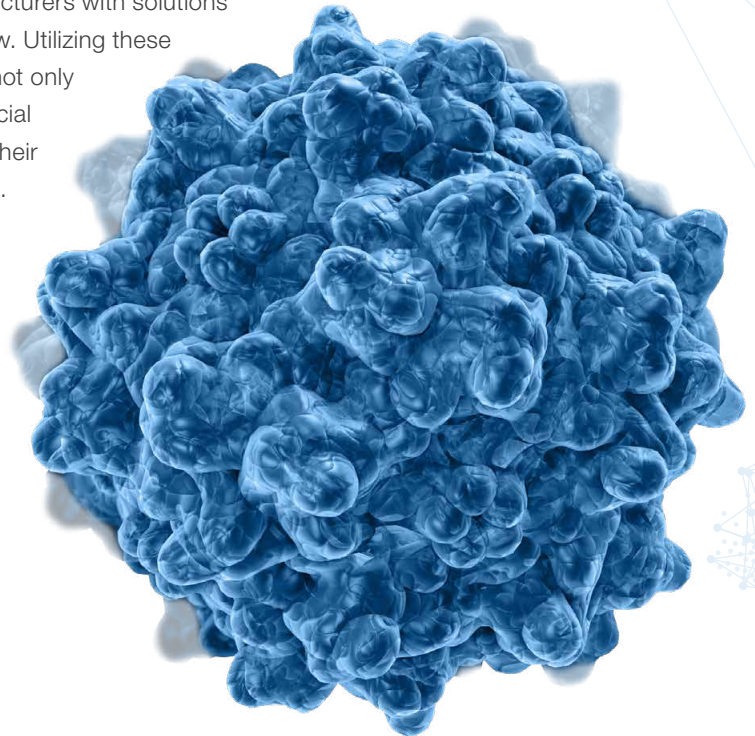
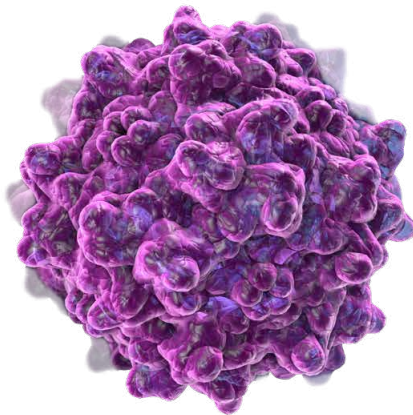
Whether they are working with a catalog or custom formulation, manufacturers can choose a format and a combination of film, tubing, fittings, and connectors suitable for easy integration into their process.



Take the next step

By combining decades of cell culture experience with cutting-edge research and development, Thermo Fisher is at the forefront of developing innovative solutions for AAV manufacturers.

From high-quality and scalable Gibco products to dedicated process development support and media manufacturing services, Thermo Fisher can provide manufacturers with solutions that span the entire AAV production workflow. Utilizing these solutions, gene therapy manufacturers can not only scale up their process from R&D to commercial manufacture with ease, but also accelerate their life-changing therapeutic's journey to market.



Learn more about Thermo Fisher's AAV manufacturing solutions at thermofisher.com/gibco-gene-therapy

gibco

Intended use of the products mentioned varies. For specific intended use statements, please refer to the product label.

© 2022 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. EXT3296 1222