DID YOU KNOW

# THERE IS A COMPLETE SYSTEM TO ENABLE PRODUCTION OF AAV VECTORS?

ThermoFisher SCIENTIFIC

## An adeno-associated viral vector production system from research to commercialization

Cost-effective, scalable adeno-associated virus (AAV) vector production is critical to meet commercial demand, and smooth scale-up to clinical production is essential. We created the Gibco™ AAV-MAX Helper-Free AAV Production System to help reduce production costs and streamline your transition from research to clinical scale.

### The AAV-MAX system features:

- **High AAV titers**—more viral particles per volume to help reduce production costs
- **Scalability**—suspension culture system with scalable protocols from shake flask to bioreactor scale
- **Simplified workflow**—streamlined, helper virus–free triple transfection protocol
- **Animal origin–free (AOF) components**—no animal- or human-derived components, to reduce raw material safety risk
- **Clonal, 293F-derived producer cells**—high-production clonal cell line; documented cGMP bank
- **Research-grade and GMP options**—seamlessly transition from discovery to commercial production

### Components of the AAV-MAX Helper-Free AAV Production System

**For research:**

- Gibco™ Viral Production Medium
- Gibco™ Viral Production Cells 2.0
- Gibco™ AAV-MAX Transfection Kit
  - Gibco™ AAV-MAX Transfection Reagent
  - Gibco™ AAV-MAX Transfection Booster
  - Gibco™ AAV-MAX Enhancer
- Gibco™ Viral-Plex™ Complexation Buffer
- Gibco™ AAV-MAX Lysis Buffer

**For clinical and commercial manufacturing:**

- Gibco™ CTS™ Viral Production Medium
- Gibco™ Viral Production Medium, AGT™
- Gibco™ CTS™ Viral Production Cells 2.0
- Gibco™ CTS™ AAV-MAX Transfection Kit
  - Gibco™ CTS™ AAV-MAX Transfection Reagent
  - Gibco™ CTS™ AAV-MAX Transfection Booster
  - Gibco™ CTS™ AAV-MAX Enhancer
- Gibco™ CTS™ Viral-Plex™ Complexation Buffer
- Gibco™ CTS™ AAV-MAX Lysis Buffer

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To help accelerate your development timelines, our scientific team has developed a complete, optimized system with integrated components to help deliver higher AAV titers than alternative platforms (Figure 1).

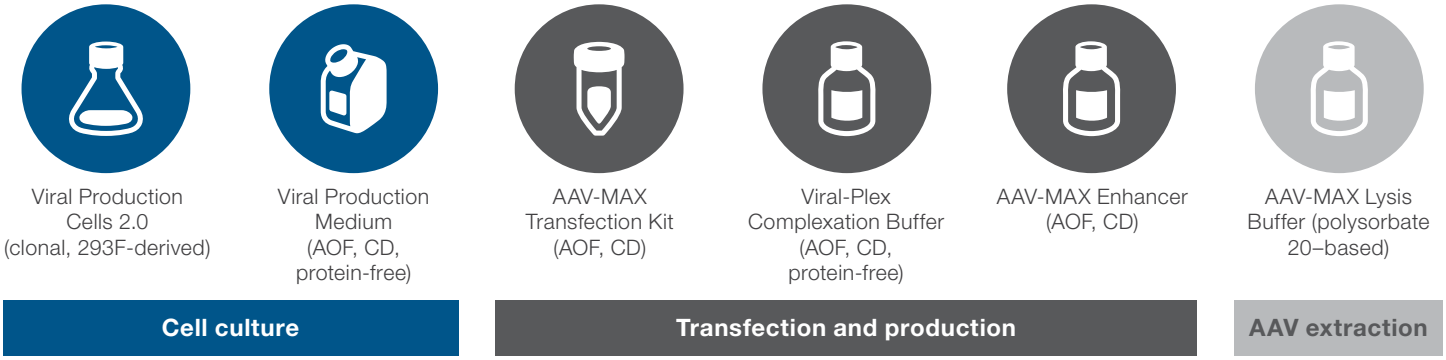


**Figure 1. Gibco™ components constituting the AAV-MAX Helper-Free AAV Production System.**

## GMP quality and regulatory support

Our products are backed by our global scale, regulatory and technical support teams, an unrelenting focus on quality, and decades of experience with good manufacturing practices (GMPs). The AAV-MAX adeno-associated viral production system is available in research grade and also as a Gibco™ CTS™ product (Gibco™ CTS™ AAV-MAX Helper-Free AAV Production System), which is manufactured in conformity with GMP for medical devices (21 CFR Part 820) and follows USP <1043>* and European Pharmacopoeia (Ph. Eur.) 5.2.12 recommendations. All CTS products come with a Drug Master File or a Regulatory Support File per customer requests associated with regulatory filings. In addition, residual test methods for the components of the AAV-MAX Transfection Kit are available upon request to support your regulatory needs.

## Optimized and fully integrated system

The components of the AAV-MAX system are designed to work synergistically, resulting in maximal titers and eliminating the need to optimize reagents and protocols. Using the complete system is more effective for producing high titers than replacing individual components or using conventional polyethyleneimine (PEI)-based transfection reagents (Figure 2).

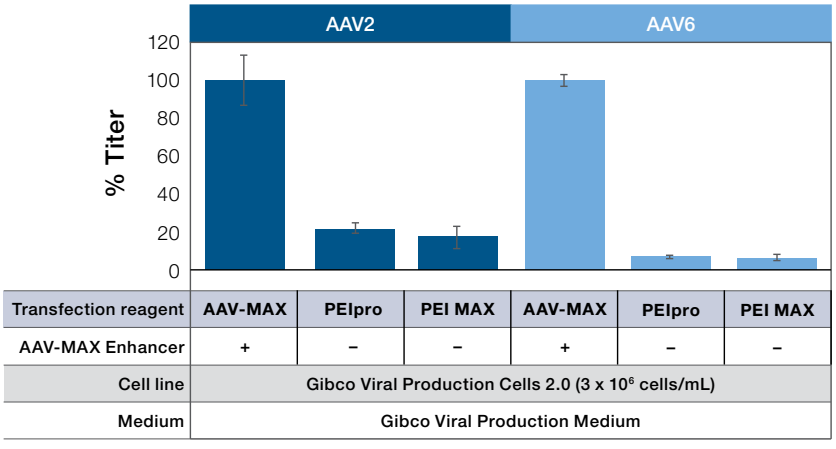


**Figure 2. A complete system for maximal performance.** AAV2 and AAV6 were produced at 30 mL scale in 125 mL shake flasks using the AAV-MAX system. The performance of the complete AAV-MAX system was evaluated and compared to that of alternative transfection reagents. Titers were measured by real-time PCR (qPCR), and the data were normalized to the titer of the complete AAV-MAX system.

* CTS products are manufactured to meet the ancillary material supplier responsibilities for cell-, gene-, and tissue-engineered products.

Other aspects of USP <1043> are the responsibility of the end user to assess.

## High titers across multiple serotypes

The innovative AAV-MAX Helper-Free AAV Production System is a complete and optimized suspension culture system that allows you to seamlessly and efficiently produce your AAV vector in high titers across multiple AAV serotypes (Figure 3).

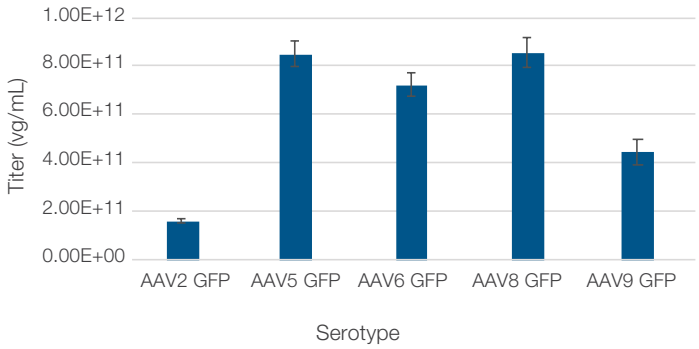


**Figure 3. The AAV-MAX system yields high titers across multiple AAV serotypes.** The system was used to produce 6 AAV serotypes at 30 mL scale in 125 mL shake flasks. Viral genome (vg) titers were measured by droplet digital PCR (ddPCR).

## Accelerate your path to the clinic

The AAV-MAX Helper-Free AAV Production System provides a smooth transition from discovery through research and commercial development manufacturing, offering one optimized, scalable platform with research-grade and GMP reagent options. Figure 4 shows the equivalence of the CTS and RUO systems with two serotypes, which would enable smooth transitions from research to cGMP-grade commercial production.

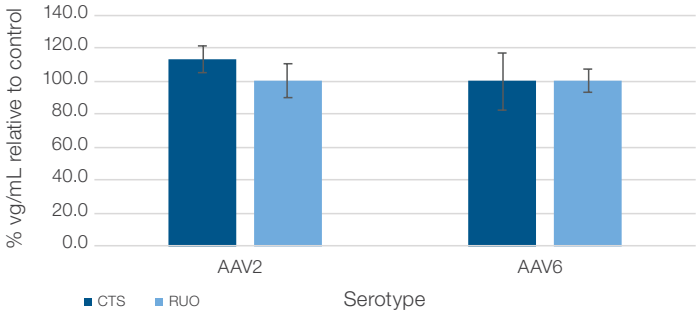


**Figure 4. Comparison of AAV titers between research-grade (RUO) and CTS reagents.** AAV serotypes AAV2 and AAV6 were produced at 30 mL production scale in 125 mL shake flasks. Performance of the RUO and CTS AAV-MAX system reagents was shown to be equivalent as measured by viral titers. Titers were measured by qPCR and data were normalized to the titer of the RUO system.

## A cost-effective AAV production solution

Get more viral particles per dollar. With the AAV-MAX system, you can obtain the same viral titer in less volume than you can with PEI-based production systems, which translates to savings in media, downstream purification, lab space, and labor. Working with smaller batches can also mitigate contamination risk. In addition, the AAV-MAX system requires less plasmid DNA per 1 x $10^6$ cells than alternative transfection reagents, which further reduces production costs.

### Switch to the AAV-MAX system and save

- Save 50% on average compared to alternative PEI-based suspension systems
- Cut plasmid DNA costs by 25%

## Scaling to meet demand

Challenges associated with existing AAV production systems include low titers, high cost of cGMP plasmid DNA, poor scalability, and a lack of fit-for-purpose cGMP manufactured reagents. The AAV-MAX system can help you overcome these challenges by allowing you to produce high AAV titers, using preoptimized and regulatory-compliant reagents developed for AAV production, in a scalable suspension platform that maintains volumetric viral titers as you scale up (Figures 5 and 6).

## Advanced dry media format option

Leave behind the concerns over classical dry media—complicated preparation, filtration difficulties, multistep processes, and lot-to-lot inconsistency—and start achieving more with the Gibco™ Advanced Granulation Technology™ (AGT™) media format.

The AGT platform provides a granular dry media format produced through a technologically advanced process that allows manufacturing of complete formulations in a variety of serum-free, protein-free, and chemically defined media. AGT granules dissolve rapidly for faster media preparation than for conventional dry powder media. Furthermore, the AGT format is efficient by nature, in that it is a complete medium that is pre-adjusted for pH and osmolality, and offers all the benefits of liquid media without the storage and transportation issues.

### Scaling in shake flasks

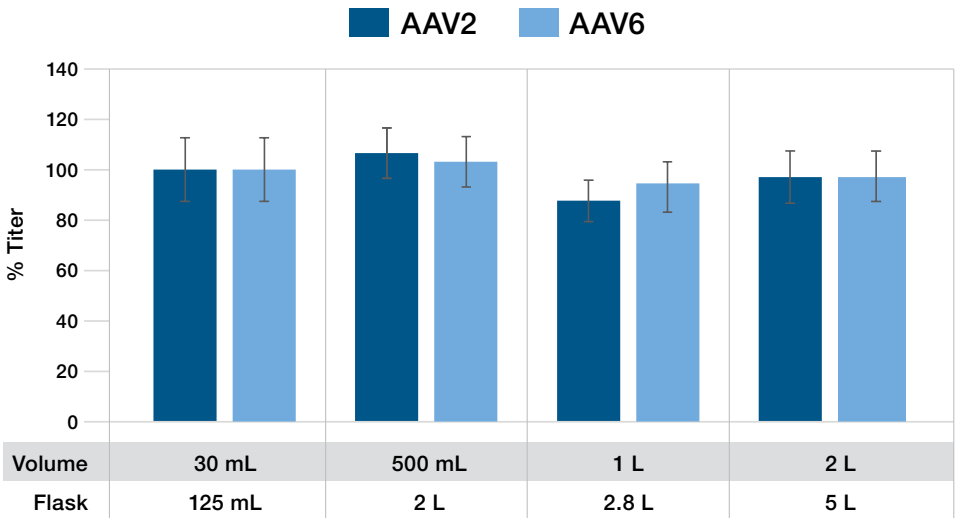


**Figure 5. The system yields high titers across multiple production scales.** The AAV-MAX system was used to produce AAV2 and AAV6 at four different scales in shake flasks. Titers were measured by qPCR, and the data were normalized to the titer at 30 mL production scale in a 125 mL shake flask.

### Scaling from shake flasks to 1,000 L single-use bioreactors

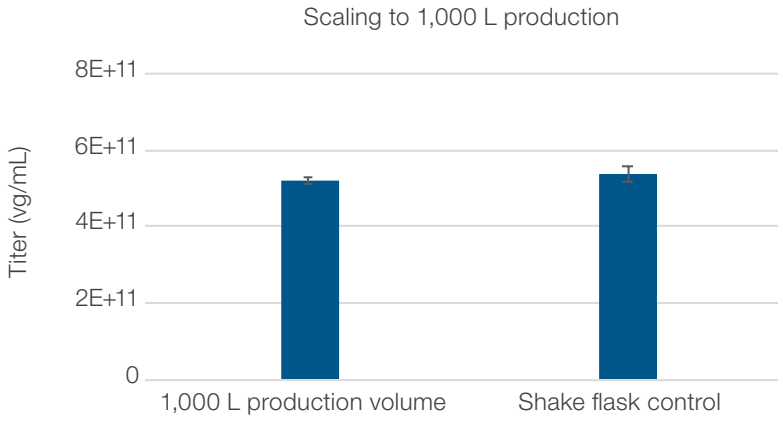


**Figure 6. High titers are achieved at a 1,000 L scale in a single-use bioreactor.** AAV6-GFP was produced using the AAV-MAX system at a 1,000 L production scale in a 5,000 L Thermo Scientific™ DynaDrive™ Single-Use Bioreactor (S.U.B.) and compared to a control produced in a shake flask. Titers were measured in crude samples with ddPCR.

## Clonal producer cell line

Viral Production Cells 2.0:

- Clonal, 293F-derived, high-producing cell line
- Optimized for high-density suspension culture (>12 x $10^6$ cells/mL) in a chemically defined medium
- No SV40 large T antigen or genetic engineering
- Robust scalability and passage stability (Figures 7–9)
- Documented, cGMP bank–manufactured as per 21 CFR 211 and EudraLex, Volume 4, and characterized as per ICH Q5A and ICH Q5D

### AAV cell line growth curve

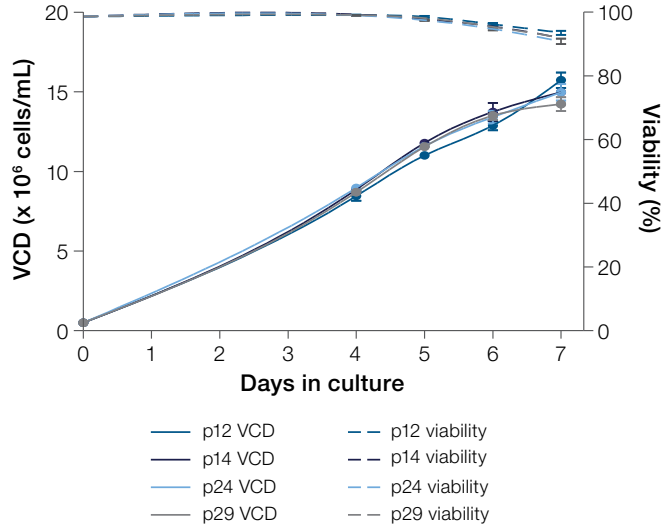


**Figure 7. Viral Production Cells 2.0 maintain similar growth profiles over multiple passages.** Cells were cultured in Viral Production Medium, and culture viability and viable cell density (VCD) were measured from day 4 to day 7 post-seeding.

### Post-thaw recovery

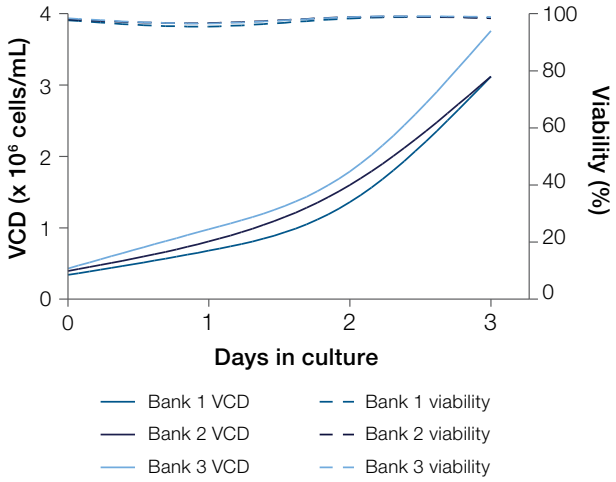


**Figure 9. Viral Production Cells 2.0 maintain good viability and viable cell density following a thaw cycle.** Cells were thawed, and culture viability and VCD were monitored for 3 days post-thaw.

### Doubling time

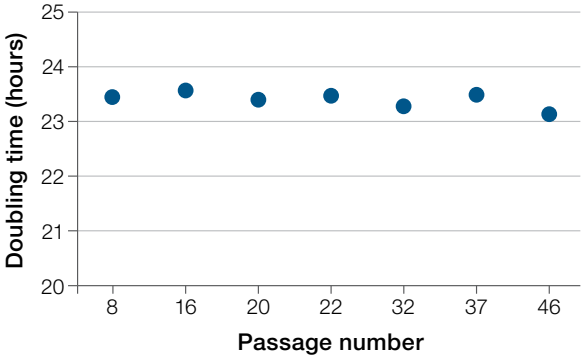


**Figure 8. Viral Production Cells 2.0 maintain a doubling time of approximately 23.5 hours over many passages.** The doubling times were calculated based on growth between days 3 and 4 post-seeding.

## Simplified workflow

To help accelerate your development timelines, our scientific team has tested hundreds of different reagent combinations to produce a complete system with preoptimized reagents that work in concert to deliver maximum titers.

### Ordering information

| Product | Quantity | Cat. No. |
|---|---|---|
| **Gibco AAV-MAX products** | | |
| AAV-MAX Helper-Free AAV Production System Kit | 1 kit | A51217 |
| Viral Production Medium | 1 L (bottle) | A4817901 |
| | 6 x 1 L (bottle) | A4817902 |
| | 10 L (bag) | A4817903 |
| Viral Production Cells 2.0 | 1 vial | A49784 |
| | 6 vials | A51218 |
| AAV-MAX Transfection Kit<br>• AAV-MAX Transfection Reagent | For 1 L culture | A50515 |
| • AAV-MAX Transfection Booster<br>• AAV-MAX Enhancer | For 10 L culture | A50516 |
| Viral-Plex Complexation Buffer | 100 mL (bottle) | A4983901 |
| AAV-MAX Lysis Buffer | 100 mL (bottle) | A50520 |
| **Gibco CTS AAV-MAX products** | | |
| CTS Viral Production Medium | 1 L (bottle) | A5144001 |
| | 6 x 1 L (bottle) | A5144002 |
| | 10 L (bag) | A5144003 |
| | 20 L (bag) | A5416001 |
| | 100 L (bag) | A5416002 |
| Viral Production Medium, AGT | 1 L | A5147101 |
| | 10 L | A5147102 |
| | 50 L | A5147103 |
| | 100 L | A5147104 |
| CTS Viral Production Cells 2.0 | 1 vial | A48400 |
| CTS AAV-MAX Transfection Kit<br>• CTS AAV-MAX Transfection Reagent | For 1 L culture | A5427701 |
| • CTS AAV-MAX Transfection Booster | For 10 L culture | A5427702 |
| • CTS AAV-MAX Enhancer | For 100 L culture | A5427703 |
| CTS Viral-Plex Complexation Buffer | 500 mL (bottle) | A5145401 |
| | 1 L (bottle) | A5145402 |
| | 10 L (bag) | A5145403 |
| CTS AAV-MAX Lysis Buffer | 100 mL (bottle) | A5152101 |
| | 1 L (bottle) | A5152102 |
| | 10 L (bag) | A5152103 |

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