AAV production and purification

Production of AAV at the 500 L scale in the DynaDrive Single-Use Bioreactor using the CTS AAV-MAX Production System

Keywords
AAV-MAX, DynaDrive S.U.B., AAV production, scale-up, single-use technology

Introduction

As the number of clinical and commercial-phase adeno-associated virus (AAV) gene therapies increases, companies are faced with the challenge of how to scale up their viral vector production processes through process development, clinical, and commercial manufacturing scales.

Prior work has demonstrated the scalability of the Gibco™ CTS™ AAV-MAX Helper-Free AAV Production System to produce AAV in the Thermo Scientific™ DynaDrive™ Single-Use Bioreactor (S.U.B.) at the 50 L scale [1,2] and 1,000 L scale [3]. As further continuation of that work, this study demonstrates scaling this same process up to an intermediate clinical manufacturing scale of a 500 L working volume in the 500 L DynaDrive S.U.B., along with specific guidance on successfully performing these processes at this scale.

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Materials and methods

Culture of CTS Viral Production Cells 2.0

Gibco™ CTS™ Viral Production Cells 2.0 (VPC 2.0) were maintained throughout the seed train and in bioreactors in Gibco™ CTS™ Viral Production Medium (Table 1). The cells were thawed and expanded in shake flasks as recommended in the AAV-MAX system user guide [4] before inoculating into the 50 L DynaDrive S.U.B. at 2 weeks post-thaw and then into the 500 L DynaDrive S.U.B. at 3 weeks post-thaw to further expand the cells prior to production. Overall, the entire seed train expansion and production run took 4 weeks to complete.

Table 1. Materials and reagents

Component	Description
CTS AAV-MAX Helper-Free AAV Production System	CTS Viral Production Cells 2.0 CTS Viral Production Medium CTS AAV-MAX Transfection Kit • CTS AAV-MAX Transfection Reagent • CTS AAV-MAX Transfection Booster • CTS AAV-MAX Enhancer CTS Viral-Plex Complexation Buffer AAV-MAX Lysis Buffer
Plasmid DNA	1.5 mg plasmid DNA per liter of culture to be transfected
Antifoaming agent	FoamAway Irradiated AOF (animal origin–free) Antifoaming Agent
Single-use bioreactor (S.U.B. hardware)	• 50 L DynaDrive S.U.B. • 500 L DynaDrive S.U.B.
Bioprocess containers (BPCs)	• DynaDrive 50 L BioProcess Container with Aegis5-14 film • DynaDrive 500 L BioProcess Container with Aegis5-14 film • 2D 5 L Labtainer BioProcess Container with Aegis5-14 film • 3D 50 L Productainer BioProcess Container with Aegis5-14 film, with outer support drum and plastic dolly
Bottle transfer assemblies and fluid transfer assembly sets	• Standard Single-Use Bottle Assembly System, 1 L size • Standard Fluid Transfer Assembly Adaptor Set, 3/8 in. ID to 1/8 in. ID • Standard Fluid Transfer Assembly 3/8 in. ID Y Manifold Set, AseptiQuik G connectors • Transfer Assembly Jumper Tubing, 3/8 in. ID, AseptiQuik G connectors

Bioreactor setup and seed train expansion

Both the 50 L and the 500 L DynaDrive S.U.B.s were set up according to the product user guide [5], and a Thermo Scientific™ HyPerforma™ G3Pro™ Bioprocess Controller was used to control each bioreactor. The operating parameters for the 500 L production culture are listed in Table 2. CTS VPC 2.0 were expanded from shake flasks into a 50 L DynaDrive S.U.B. at a working volume of 10 L and a starting density of 3.5×10^5 cells/mL, which was then brought up to a working volume of 45 L and a starting density of 6.0×10^5 cells/mL after 3 days. Once the cells reached a density of at least 4.5×10^6 cells/mL, the culture was used to seed the 500 L DynaDrive S.U.B. at a 150 L working volume and starting density of 6.0×10^5 cells/mL as an N-1 culture. After 3 days, the culture was brought up to a 450 L working volume and a target seed density of 1.5×10^6 viable cells per mL (VC/mL) for the production run. To prepare the cultures for transfection, CTS VPC 2.0 were grown to a target density of at least 3.0×10^6 ($\pm 0.5 \times 10^6$) VC/mL with ≥95% viability within 24 hours of inoculating. Control cultures were grown in shake flasks throughout scale-up and production. Growth kinetics, metabolic profiles, viral genome and infectious titers, and percentage of full capsids were measured and compared to the corresponding bioreactor data.

Table 2. Bioreactor parameters

Parameter	Condition in 500 L DynaDrive S.U.B.
Final working volume	500 L
Temperature	37°C
pH	7.0 ± 0.2
pH control	CO_2 through the drilled-hole sparger (DHS)
Agitation	70 rpm, P/V: 20.6 W/m^3
Dissolved oxygen (DO)	40%
DO cascade	Air supplemented with oxygen through the DHS
Headspace aeration	7 slpm
Target seeding density	1.5×10^6 VC/mL

Transfection of bioreactor cultures

The reagent volumes recommended for transfecting a 450 L culture are listed in Table 3. All reagents were maintained at 4–8°C prior to complexation of the transfection reagent and plasmid DNA. The transfer plasmid DNA, packaging plasmid DNA, and helper plasmid DNA were combined into a 1 L PETG bottle. The complexation mixture was then transferred to a 3D Thermo Scientific™ Productainer™ BPC (housed in a barrel) containing Gibco™ CTS™ Viral-Plex™ Complexation Buffer at 4–8°C, by replacing the lid of the bottle with the 2-port cap of the Thermo Scientific™ Standard Single-Use Bottle Assembly System cap and using a Thermo Scientific™ Standard Fluid Transfer Assembly Adaptor Set to allow for the tubing to step down from the 3/8 in. ID size to the 1/8 in. ID size used in the bottle assembly. Once connected, the plasmid was added to the Viral-Plex Complexation Buffer via gravity feed over a period of 3 minutes. Separately, Gibco™ CTS™ AAV-MAX Transfection Booster and Gibco™ CTS™ AAV-MAX Transfection Reagent, which were both in Thermo Scientific™ Labtainer™ BPCs with AseptiQuik™ G connectors, were combined by connecting an end of a Thermo Scientific™ Standard Fluid Transfer Assembly Y Set to each Labtainer BPC via the AseptiQuik G connectors and adding the booster into the reagent Labtainer BPC. The combined BPC was mixed by gently rocking back and forth by hand to create a wave motion for 5–10 seconds, then allowed to sit and incubate for 10 minutes. The mixture was then transferred into the Productainer BPC containing the diluted DNA by welding the third end of the Y set to the Productainer BPC and gravity-draining the booster/reagent mix into the Productainer BPC over a period of 2 minutes. The Productainer BPC/barrel was moved from side to side on casters for 5–10 seconds, then incubated at room temperature without further agitation, as it was sterile-welded onto a sterile Levitronix™ single-use pump head irradiated with 3/8 in. ID line sets on the inlet and outlet and then connected via an AseptiQuik G connector to the probe belt at the base of the 500 L DynaDrive S.U.B to allow for a subsurface addition. After 20 minutes of incubation, the Levitronix single-use pump was used to transfer the solution into the bioreactor at a rate of approximately 10 L/min. The complex was added to the bioreactor over a period of 6 minutes. A Labtainer BPC containing Gibco™ CTS™ AAV-MAX Enhancer was sterile-welded to a feed line of the bioreactor after transfection, and the enhancer was added to the system via gravity feed over a period of 5 minutes.

Culture monitoring

Samples were collected daily to assess the growth rate and health of the cells. Approximately 15 mL was pulled from the bioreactor and discarded to flush the sampling lines. Fresh 20 mL samples were then collected to measure pH, pO_2, pCO_2, and nutrient and metabolite levels using a BioProfile™ FLEX2 Automated Cell Culture Analyzer (Nova Biomedical). A Vi-CELL™ XR Cell Viability Analyzer (Beckman Coulter) was used to evaluate the concentration and viability of the cells, and an Oakton™ pH 150 Meter (Cole-Parmer) was used for additional offline pH measurements. The remaining volumes were then dispensed into 1 mL vials and frozen at –80°C for titer analysis. Gibco™ FoamAway™ antifoaming agent was added to the bioreactor cultures as needed to minimize foaming. The 50 L DynaDrive S.U.B. used just over 9 mL over the course of the 10 L and 45 L culture, and the 500 L DynaDrive S.U.B. used less than 150 mL over the course of in-vessel expansion and the production run.

Table 3. Recommended reagent volumes for a 500 L production run.

Reagent	Volume required per liter of culture	Volume added to the bioreactor
Volume of culture to be transfected	1 L	500 L
Total plasmid DNA	1.5 mg	0.675 L*
CTS Viral-Plex Complexation Buffer	100 mL	45 L
CTS AAV-MAX Transfection Reagent	6 mL	2.7 L
CTS AAV-MAX Transfection Booster	3 mL	1.35 L
CTS AAV-MAX Enhancer	10 mL	4.5 L
Final culture volume	1.11 L	~500 L

* Assuming stock plasmid DNA concentration of 1 mg/mL.

Quantitation of transfection efficiency, viral genome titers, and infectivity titers

Transfection efficiency was quantified by performing a GFP-fluorescing cell count versus a total cell count on a Cellometer™ K2 Fluoresecnt Cell Counter (Nexcelom). Transfection efficiencies were determined 24 hours after transfection.

For titer determination via droplet digital PCR (ddPCR), samples were collected and frozen at –80°C at different time points leading up to and at the time of harvest. Genome titers were determined using primers and probes that targeted the GFP gene sequence. Prior to ddPCR analysis, cells were lysed and samples were subjected to nuclease treatment to remove non-encapsidated DNA. Following nuclease treatment, viral genome titers were determined by ddPCR using serial dilutions of each sample.

Viral infectivity titers were determined by inoculating HT1080 cells with serial dilutions of AAV cell lysates and incubating them at 37°C for 48 hours. After the incubation period, transduction units per mL (TU/mL) for each sample was calculated based on the number of GFP-positive cells per well as determined by flow cytometry.

The percentage of full and empty capsids was determined by total capsid ELISA in conjunction with ddPCR, as shown in the equation:

$$\text{Full capsids (\%)} = \frac{\text{ddPCR titer}\left(\frac{\text{vg}}{\text{mL}}\right)}{\text{Capsid titer}\left(\frac{\text{capsids}}{\text{mL}}\right)}$$

Results

Cell densities and viabilities trended closely between the 125 mL shake flask controls and the 500 L DynaDrive S.U.B. during seed train preparation and AAV6-GFP production (Figure 1). Glucose, lactate, and ammonia levels were also comparable between the bioreactor and control flasks throughout the production run, with ammonia levels remaining low overall (Figures 2–4).

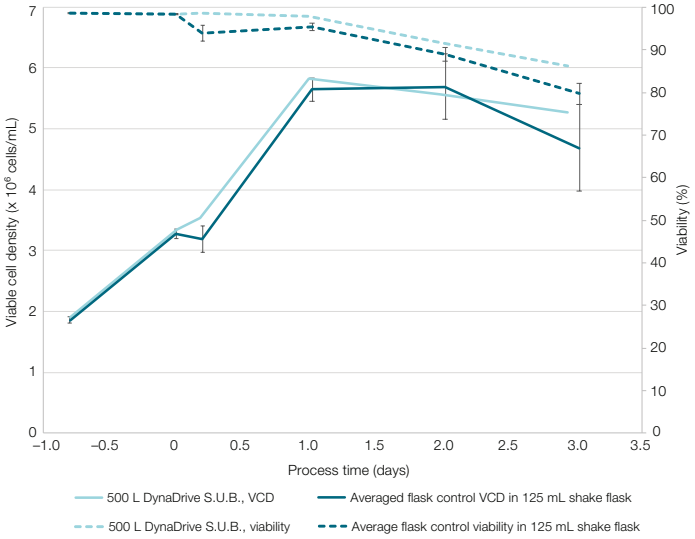

Figure 1. Growth profiles of CTS VPC 2.0 during AAV6-GFP production in 125 mL shake flasks and the 500 L DynaDrive S.U.B. Day 0 is the day of transfection. Harvest was 3 days later.

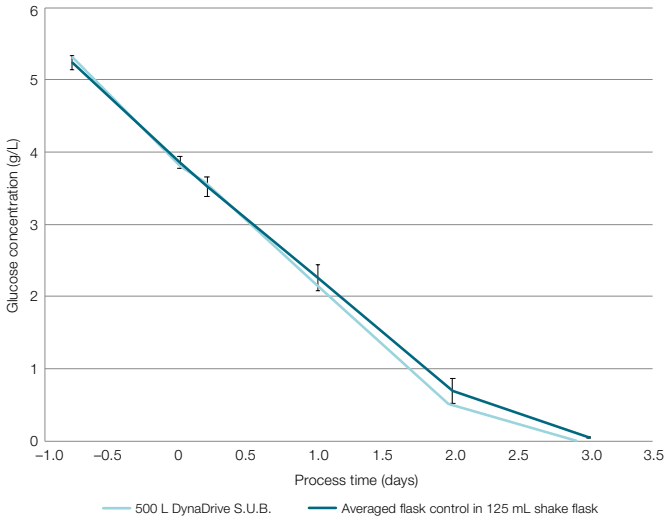

Figure 2. Glucose profiles of CTS VPC 2.0 during AAV6-GFP production in 125 mL shake flasks and the 500 L DynaDrive S.U.B. Day 0 is the day of transfection. Harvest was 3 days later.

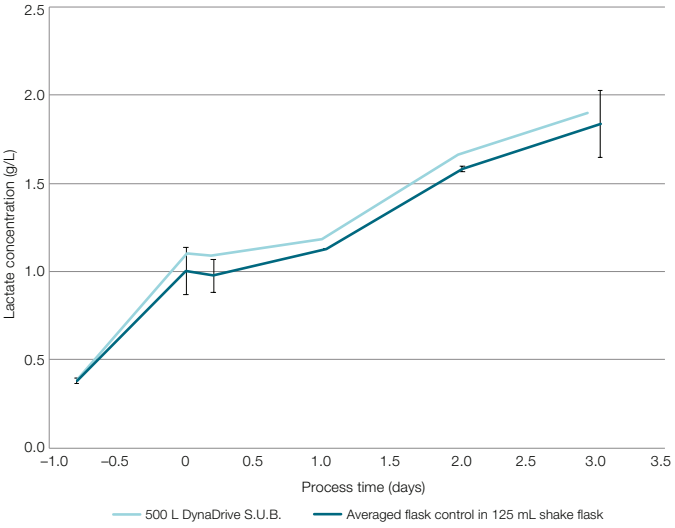

Figure 3. Lactate profiles of CTS VPC 2.0 during AAV6-GFP production in 125 mL shake flasks and the 500 L DynaDrive S.U.B. Day 0 is the day of transfection. Harvest was 3 days later.

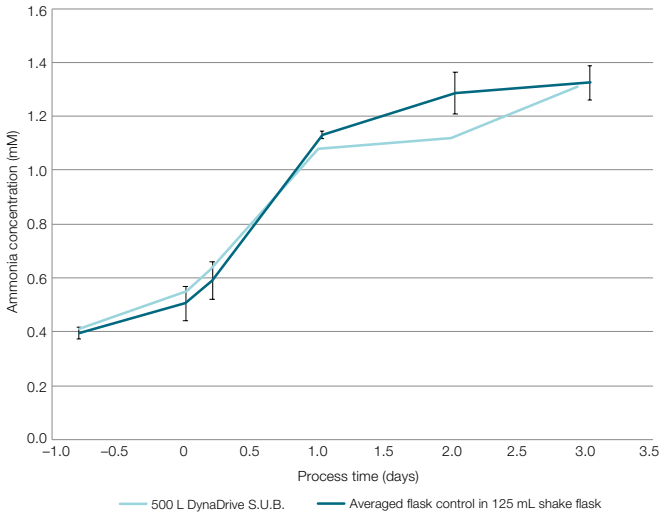

Figure 4. Ammonia profiles of CTS VPC 2.0 during AAV6-GFP production in 125 mL shake flasks and the 500 L DynaDrive S.U.B. Day 0 is the day of transfection. Harvest was 3 days later.

Transfection efficiencies 24 hours post-transfection trended higher in the 500 L DynaDrive S.U.B. than in control flasks (Figure 5). The genomic infectious titer results were equivalent between the control flasks and the S.U.B. (Figures 6, 7). Lastly, the percentage of full capsids was higher in the S.U.B. than in the shake flasks (Figure 8).

Taken together, the positive trends in AAV productivity observed in the DynaDrive S.U.B.s suggest that the additional control of process parameters may allow for improved AAV productivity and quality attributes. As such, additional optimization of bioreactor parameters is warranted to further enhance the performance of the AAV-MAX system in the bioreactor setting.

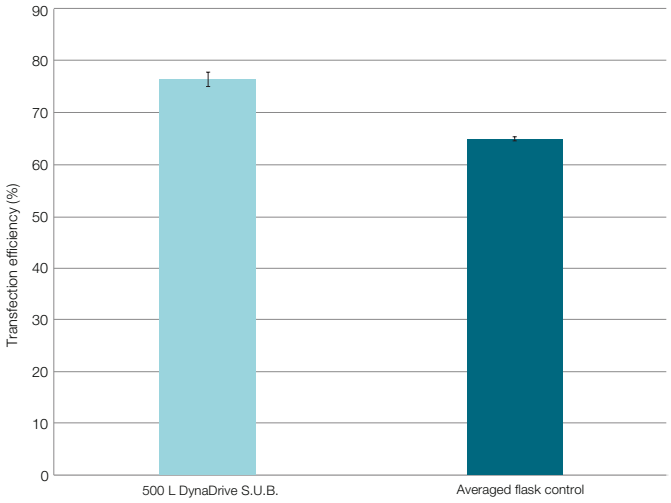

Figure 5. Transfection efficiencies of CTS VPC 2.0 at 24 hours post-transfection during AAV6-GFP production in the 500 L DynaDrive S.U.B. and 125 mL shake flasks. Transfection efficiency is calculated from the number of are cells exhibiting GPF fluorescence compared to the total cell count. Error bars indicate the standard deviation of averaged duplicate samples.

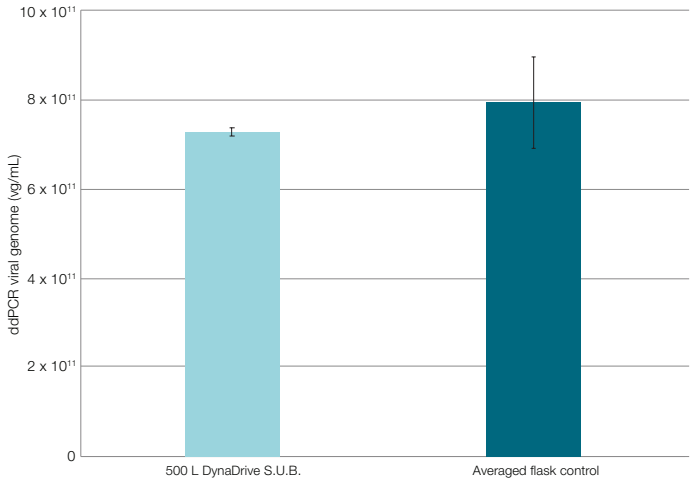

Figure 6. Viral genome titers of the crude harvest of CTS VPC 2.0 during AAV6-GFP production in the 500 L DynaDrive S.U.B. and 125 mL shake flasks. Error bars indicate the standard deviation of averaged duplicate samples.

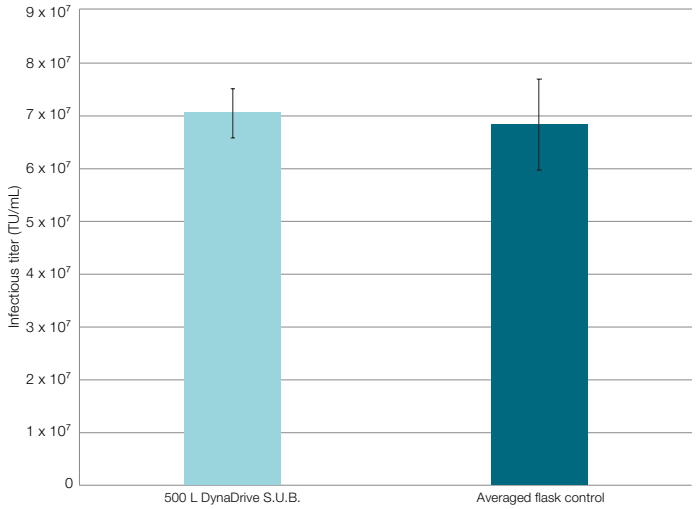

Figure 7. Infectious titers of the crude harvest of CTS VPC 2.0 during AAV6-GFP production in the 500 L DynaDrive S.U.B. and 125 mL shake flasks. Error bars indicate the standard deviation of averaged duplicate samples.

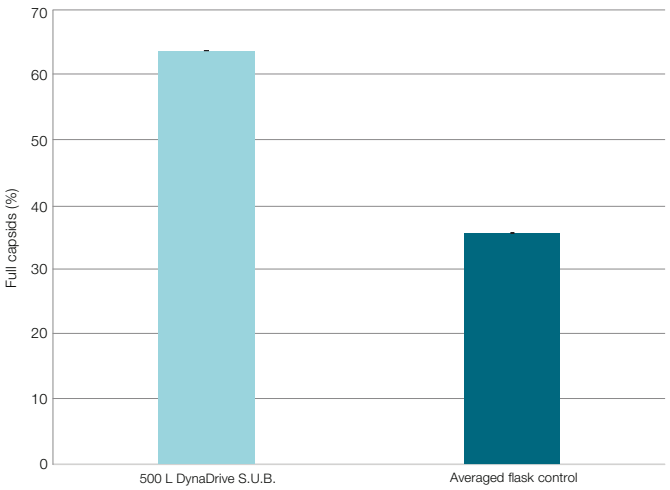

Figure 8. Percentage of full capsids in the crude harvest of CTS VPC 2.0 during AAV6-GFP production in the 500 L DynaDrive S.U.B. and 125 mL shake flasks. Error bars indicate the standard deviation of averaged duplicate samples.

Conclusions

The CTS AAV-MAX Production System enables scalable AAV production from research and process development through clinical and commercial manufacturing. Additionally, the DynaDrive S.U.B. helps simplify and optimize the operating parameters for scaling up to clinical and commercial manufacturing. In this study, the 500 L DynaDrive S.U.B. yielded AAV6 titers that were comparable to those obtained in shake flasks, and similar to those of the 50 L DynaDrive S.U.B. obtained in work done previously [1,2]. In-vessel cell expansion in both the 50 L DynaDrive S.U.B. and the 500 L DynaDrive S.U.B. reduced the complexity of the seed train workflow by eliminating the need for intermediate vessels, consumables, and operating space. It also increased the efficiency of the seed train itself by reducing the number of connections and transfer loss.

Scale-up for AAV production in the 500 L DynaDrive S.U.B. was achieved by choosing simple scale-up parameters based on the previous work with the CTS AAV-MAX AAV Production System in the 50 L DynaDrive S.U.B. [1,2]. The gas flow and agitation rates tested in this study allowed sufficient O_2 mass transfer to maintain the required DO set point without shear damage to the cells or excessive foaming.

These results highlight the robust scalability of both the CTS AAV-MAX Helper-Free AAV Production System and the DynaDrive S.U.B. to help meet current and future demands of gene therapy researchers.

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4. AAV-MAX Helper-Free Production System User Guide. Thermo Fisher Scientific, September 2021.
5. DynaDrive S.U.B. User's Guide. Thermo Fisher Scientific, June 2021.

Appendix

Protocol for 500 L working volume S.U.B. with an example of seed train expansion

1. Subculture and expand CTS Viral Production Cells 2.0 (VPC 2.0) until they reach a density of 4 x 10^6 to 6 x 10^6 viable cells per mL (VC/mL) in a culture volume that will yield 3 x 10^9 to 6 x 10^9 cells to inoculate the 50 L DynaDrive S.U.B. or Thermo Scientific™ HyPerforma™ 5:1 S.U.B. at a working volume of 10 L. This will yield a starting density of 0.3 x 10^6 to 0.6 x 10^6 VC/mL, depending on if you are performing a 4-day or a 3-day passage, respectively.

2. Prepare the DynaDrive S.U.B. or the HyPerforma 5:1 S.U.B. prior to inoculation as follows:

   a. Two days prior to inoculation, load the BPC into the hardware, clamping all lines and ports not in use.

   b. Prior to filling the S.U.B. with medium, the BPC can be purged with N_2 or air, if desired.

   c. If single-use dissolved oxygen (DO) and pH sensors are not built into the S.U.B., prepare and autoclave sensors, using probe assemblies, and insert them into the S.U.B. prior to addition of medium.

   d. One day prior to inoculation, add 8 L of CTS Viral Production Medium (VPM) to the S.U.B.

   e. Turn on agitation for the S.U.B. and heat to 37°C while N_2 is sparged into the system. Once the reactor is warmed and the DO reading has stabilized, begin calibration of the DO probe, starting with the zero point. Then, turn off the N_2 and turn on air sparging and proceed to calibrate the span for DO.

   f. Once the DO probe is calibrated, turn on cascade loops for the run to equilibrate the system, and pull an offline sample to check the pH calibration. If using a single-use pH sensor, input the calibration information into the controller, then perform a 1-point offset, if required.

3. Once cells reach a density of 4 x 10^6 to 6 x 10^6 VC/mL in the shake flask, inoculate the prepared DynaDrive S.U.B. or HyPerforma 5:1 S.U.B. by adding CTS VPC 2.0 from step 1 to a final density of 0.3 x 10^6 to 0.6 x 10^6 VC/mL (for a 4-day or 3-day passage, respectively) in a final working volume of 10 L. This will be the N-3 culture. Set points for the N-3 reactors are in Table 4.

4. When the cells reach 4 x 10^6 to 6 x 10^6 VC/mL, add fresh CTS VPM to the culture, bringing the working volume up to 50 L and the cell density to 0.3 x 10^6 to 0.6 x 10^6 VC/mL, depending on if it is a 4-day or 3-day passage, respectively. This will be the N-2 culture. Adjust the set points for the reactor as shown in Table 5.

Table 4. Recommended set points for N-3 culture in 50 L S.U.B.s.

Parameter	10 L WV in 50 L DynaDrive S.U.B.	10 L WV in 50 L 5:1 S.U.B.
Temperature	37°C ± 0.5°C	37°C ± 0.5°C
Working volume (WV)	10 L	10 L
Sparger	Enhanced DHS	DHS
Impellers at WV	Bottom sweep impeller, 1 fully covered 2-bladed pitch impeller	Three-bladed pitch impeller
Impeller diameter	4.28 in.	4.37 in.
Agitation	90 rpm, P/V 17.3 W/m^3, tip speed 0.51 m/sec	97 rpm, P/V 15 W/m^3, tip speed 0.56 m/sec
Headspace gassing	Air: 1.0 slpm, through crossflow sparge	Air: 1.0 slpm, through crossflow sparge
Dissolved oxygen (DO)	40%, controlled by air (0–0.25 slpm) and O_2 (0–1 slpm)	40%, controlled by O_2 (0–1 slpm)
pH	7.0 ± 0.2, controlled by CO_2 (0–1 slpm)	7.0 ± 0.2, controlled by CO_2 (0–1 slpm)

Table 5. Recommended set points for N-2 culture in 50 L S.U.B.s.

Parameter	50 L WV in 50 L DynaDrive S.U.B.	50 L WV in 50 L 5:1 S.U.B.
Temperature	37°C ± 0.5°C	37°C ± 0.5°C
Working volume (WV)	50 L	50 L
Sparger	Enhanced DHS	DHS
Impellers at WV	Bottom sweep impeller, 3 fully covered 2-bladed pitch impellers	Three-bladed pitch impeller
Impeller diameter	4.28 in.	4.37 in.
Agitation	95 rpm, P/V 15.6 W/m^3, tip speed 0.54 m/sec	166 rpm, P/V 15 W/m^3, tip speed 0.97 m/sec
Headspace gassing	Air: 1.5 slpm	Air: 1.5 slpm
Dissolved oxygen (DO)	40%, controlled by air (0–0.25 slpm) and O_2 (0–1 slpm)	40%, controlled by O_2 (0–1 slpm)
pH	7.0 ± 0.2, controlled by CO_2 (0–1 slpm)	7.0 ± 0.2, controlled by CO_2 (0–1 slpm)

5. Prepare the 500 L DynaDrive S.U.B. or the 500 L HyPerforma 5:1 S.U.B. to be used for the N-1 and N cultures, prior to inoculation as follows:

   a. Two days prior to inoculation, load the BPC into the hardware, clamping all lines and ports not in use.

   b. Prior to filling the S.U.B. with medium, the BPC can be purged with N_2 or air, if desired.

   c. If single-use DO and pH sensors are not built into the S.U.B., prepare and autoclave sensors, using probe assemblies, and insert them into the S.U.B. prior to the addition of medium.

   d. One day prior to inoculation, add 125 L of CTS VPM to the S.U.B.

   e. Turn on agitation for the S.U.B. and heat to 37°C while N_2 is sparged into the system. Once the reactor is warmed and the DO reading has stabilized, begin calibration of the DO probe, starting with the zero point. Then, turn off the N_2, turn on air sparging, and proceed to calibrate the span for DO.

   f. Once the DO probe is calibrated, turn on cascade loops for the run to equilibrate the system, and pull an offline sample to check the pH calibration. If using a single-use pH sensor, input the calibration information into the controller, then perform a 1-point offset, if required.

6. Once cells reach a density of 4 x 10^6 to 6 x 10^6 VC/mL in the 50 L S.U.B., inoculate the prepared DynaDrive S.U.B. or HyPerforma 5:1 S.U.B. by adding CTS VPC 2.0 from step 4 to a final density of 0.3 x 10^6 to 0.6 x 10^6 VC/mL (for a 4-day or 3-day passage, respectively) in a final working volume of 150 L. This will be the N-1 culture. Set points for the N-1 reactors are in Table 6.
   Note: This seed train expansion protocol (steps 1–6) is an example of how to expand these cells into large-scale bioreactors and can be modified as needed, depending on your process. For best results during your production process, it is important to passage and expand these cells while they are between 4 x 10^6 and 6 x 10^6 VC/mL.

7. When the cells reach 4 x 10^6 to 6 x 10^6 VC/mL, add fresh CTS VPM to the culture to inoculate the production run at 1.5 x 10^6 VC/mL in 450 L. Adjust the set points for the reactor as shown in Table 7.

Table 6. Recommended set points for N-1 culture in 500 L S.U.B.s.

Parameter	150 L WV in 500 L DynaDrive S.U.B.	150 L WV in 500 L 5:1 S.U.B.
Temperature	37°C ± 0.5°C	37°C ± 0.5°C
Working volume (WV)	150 L	150 L
Sparger	Enhanced DHS	DHS
Impellers at WV	Bottom sweep impeller, 1 fully covered and 1 partially covered 2-bladed pitch impeller	Three-bladed pitch impeller
Impeller diameter	9.03 in.	9.85 in.
Agitation	68.1 rpm, P/V 20.0 W/m^3, tip speed 0.82 m/sec	67.7 rpm, P/V 20.0 W/m^3, tip speed 0.89 m/sec
Headspace gassing	Air: 7.0 slpm	Air: 7.0 slpm
Dissolved oxygen (DO)	40%, controlled by air (0–2.5 slpm) and O_2 (0–10 slpm)	40%, controlled by O_2 (0–10 slpm)
pH	7.0 ± 0.2, controlled by CO_2 (0–1 slpm)	7.0 ± 0.2, controlled by CO_2 (0–1 slpm)

Table 7. Recommended set points for the production run in 500 L S.U.B.s.

Parameter	450–500 L WV in 500 L DynaDrive S.U.B.	450–500 L WV in 500 L 5:1 S.U.B.
Temperature	37°C ± 0.5°C	37°C ± 0.5°C
Working volume (WV)	500 L post-transfection	500 L post-transfection
Sparger	Enhanced DHS	DHS
Impellers at WV	Bottom sweep impeller, 3 fully covered 2-bladed pitch impellers	Three-bladed pitch impeller
Impeller diameter	9.03 in.	9.85 in.
Agitation	69.2 rpm, P/V 20.0 W/m^3, tip speed 0.83 m/sec	101.1 rpm, P/V 20.0 W/m^3, tip speed 1.33 m/sec
Headspace gassing	Air: 7.0 slpm	Air: 7.0 slpm
Dissolved oxygen (DO)	40%, controlled by air (0–2.5 slpm) and O_2 (0–10 slpm)	40%, controlled by O_2 (0–10 slpm)
pH	7.0 ± 0.2, controlled by CO_2 (0–2 slpm)	7.0 ± 0.2, controlled by CO_2 (0–2 slpm)

8. On the day of transfection, determine viability and viable cell density. We recommend reaching 3.0 x 10^6 ± 0.5 x 10^6 VC/mL and ≥95% viability before proceeding with transfection.

9. Prepare the transfer plasmid DNA, packaging plasmid DNA, and helper plasmid DNA in your optimized ratio. Use 1.5 mg total plasmid DNA per L of culture volume for transfection (Table 8).
Note: This ratio should be optimized based on plasmid size and serotype.

10. Prepare the CTS AAV-MAX Transfection Reagent and plasmid DNA complexes as indicated in Table 8.
Note: All reagents for the complexation reaction should be kept at 2–8°C until use and should be cold at the time of use.

    a. Mix the Labtainer BPC containing CTS AAV-MAX Transfection Reagent by gently rocking back and forth by hand to create a wave motion for approximately 5–10 seconds.

    b. Combine the required amounts of the CTS AAV-MAX Transfection Reagent and CTS AAV-MAX Transfection Booster into a 5 L Labtainer BPC.

    c. Mix the Labtainer BPC by gently rocking back and forth by hand to create a wave motion for 5–10 seconds to mix the contents, and incubate at room temperature for 10 minutes without further agitation.

    d. Separately, to a 50 L Productainer BPC in a barrel on a drum dolly containing 10% of the volume of culture to be transfected of cold CTS Viral-Plex Complexation Buffer, add the total plasmid DNA from step 9 by adding a single-use bottle assembly to the bottle containing the DNA and sterile-welding it onto the BPC, adding a fluid transfer adapter tubing set, if required, to step down in tubing size. The DNA can be pumped or added via gravity draining.

    e. Mix the Productainer BPC by gently pushing the barrel back and forth for about 5–10 seconds, then incubate without further agitation while the next steps are performed.

    f. Connect the Labtainer BPC containing the CTS AAV-MAX Transfection Booster and CTS AAV-MAX Transfection Reagent from step 10b to the diluted plasmid DNA from step 10d by sterile-welding it onto the Productainer BPC. The contents of the Labtainer BPC can then be added via gravity draining quickly into the BPC.

    g. Mix the Productainer BPC by gently pushing the barrel back and forth for about 5–10 seconds, then incubate for 20 minutes without further agitation. During incubation, sterile-weld the Productainer BPC to a prepared pump line set that has been connected to the bioreactor through a standard feed line at the top of the BPC or an optional submerged feed line on the probe belt, utilizing a transfer assembly jumper tubing line set connected via AseptiQuik G connectors to create this submerged feed line. After incubation, use either a peristaltic pump (one or more) or a Levitronix pump to transfer the solution to the bioreactor at a rate of ≥5 lpm, if possible. If not possible, utilize a pump rate that will ensure that the complexation mixture is transferred into the bioreactor as quickly as possible.

    Note: 1. For optimal complexation, minimize agitation of the Productainer and Labtainer BPCs to the movements that are necessary so as to not disrupt the complexation reaction.
    2. For optimal plasmid DNA complexation at larger scales, we recommend using cold reagents and complexing for a minimum of 20 minutes before addition to the bioreactor. The complexation mixture should be added to the bioreactor within 60 minutes following initiation of the complexation reaction.

11. Add a volume of CTS AAV-MAX Enhancer to the bioreactor that is equal to 1% of the volume that was transfected (Table 8) by welding the Labtainer BPC containing the enhancer to a feed line at the top of the bioreactor BPC and gravity-draining or pumping the enhancer into the bioreactor.

Table 8. Recommended transfection parameters.

Reagent	Amount required per liter of culture to transfect	Volume to be added to 450 L reactor
Volume of culture to be transfected	450 L	
Total plasmid DNA	1.5 mg	675 mL*
CTS Viral-Plex Complexation Buffer	100 mL	45 L
CTS AAV-MAX Transfection Reagent	6 mL	2.7 L
CTS AAV-MAX Transfection Booster	3 mL	1.35 L
CTS AAV-MAX Enhancer	10 mL	4.5 L
Final culture volume	~500 L	

* Assuming stock plasmid DNA concentrations of 1 mg/mL.

12. Harvest cultures approximately 72 hours post-transfection (optimal harvest times will depend on the particular AAV construct). Lyse cells by adding 10X AAV-MAX Lysis Buffer to the bioreactor to a final concentration of 1X. Additionally, add $MgCl_2$ and Thermo Scientific™ Pierce™ Universal Nuclease to final concentrations of 2 mM and 90 U/mL, respectively, to digest any non-encapsidated nucleic acids (Table 9). Agitate the reactors at 37°C for at least 2 hours without gassing to complete the lysis and nucleic acid digestion step.

Recommendations for scaling to larger vessels

It is important to consider the power input per volume (P/V), tip speed, mixing time, and addition of the transfection complex. The P/V in the protocol is approximately 20 W/m^3, and the tip speed is 0.82–1.33 m/sec, depending on the type of S.U.B. used. This provides a robust system at the 1,000 L working volume that minimizes shear from the impeller while still providing optimal mixing. We recommend scaling up based on P/V when possible.

Pay attention to the hold time and mixing of transfection reagents, DNA, and the transfection complex. Ensure optimal mixing at each step and that incubation and the duration of addition is not prolonged.

Table 9. Recommended lysis and nuclease treatment conditions.

Reagent	Volume	Final concentration in reactor
Final production volume	500 L	100%
AAV-MAX Lysis Buffer (10X)	50 L	1X
$MgCl_2$	1 L*	2 mM
Pierce Universal Nuclease	180 mL**	90 U/mL
Final harvest volume	550 L	

* Assuming a 1 M stock concentration of $MgCl_2$.

** Assuming a stock concentration of 250,000 U/mL.

Ordering information

Product	Description	Cat. No.
AAV-MAX products		
AAV-MAX Helper-Free AAV Production System Kit	1 kit	A51217
Viral Production Medium	1 L	A4817901
	6 x 1 L	A4817902
	10 L (bag)	A4817903
Viral Production Cells 2.0	1 vial	A49784
	6 vials	A51218
AAV-MAX Transfection Kit • AAV-MAX Transfection Reagent • AAV-MAX Transfection Booster • AAV-MAX Enhancer	For 1 L culture	A50515
	For 10 L culture	A50516
Viral-Plex Complexation Buffer	100 mL	A4983901
AAV-MAX Lysis Buffer	100 mL	A50520
CTS AAV-MAX products		
CTS Viral Production Medium	1 L	A5144001
	6 x 1 L	A5144002
	10 L	A5144003
	20 L	A5416001
	100 L	A5416002
Viral Production Medium, AGT format	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 • CTS AAV-MAX Transfection Reagent • CTS AAV-MAX Transfection Booster • CTS AAV-MAX Enhancer	For 1 L culture	A5427701
	For 10 L culture	A5427702
	For 100 L culture	A5427703
CTS Viral-Plex Complexation Buffer	For 5 L production	A5145401
	For 10 L production	A5145402
	For 100 L production	A5145403

Ordering information (continued)

Product	Description	Cat. No.
Additional upstream AAV production products		
FoamAway Irradiated AOF (animal origin–free) Antifoaming Agent	0.5 L in a 1 L bag	A1036902
Pierce Universal Nuclease for Cell Lysis	100 kU	88702
Bioprocess equipment and consumables		
DynaDrive Single-Use Bioreactor, 120 V	50 L	DDB0050.1011
	500 L	DDB0500.1011
DynaDrive Single-Use Bioreactor, 240 V	3,000 L	DDB3000.1021
	5,000 L	DDB5000.1021
HyPerforma G3Pro Bioprocess Controller	For 50 L and 500 L DynaDrive S.U.B. and 5:1 S.U.B.s	F100-2961-001
	For 3,000 L/5,000 L DynaDrive S.U.B.	F100-2061-100
DynaDrive BioProcess Container	50 L	SH31192.01
	500 L	SH31193.01
	3,000 L	SH31196.01
	5,000 L	SH31195.01
2D Labtainer BioProcess Container	5 L	SH30712.01
	10 L	SH30712.02
2D Labtainer Pro BioProcess Container	5 L	PL30023.12
	10 L	PL30023.13
3D Productainer BioProcess Container, 2 Top Ports	50 L	SH30964.01
3D Productainer BioProcess Container, 2 Top Ports and 1 Bottom Port	50 L	SH30967.01
3D Productainer BioProcess Container, 4 Top Ports	50 L	SH30966.01
3D Productainer BioProcess Container, 4 Top Ports and 1 Bottom Port	50 L	SH30969.01
Flat-bottom linear low-density polyethylene (LLDPE) drum, top dispense, with clamps	50 L	SV50076.02
Conical LLDPE drum, 1 port, size 10.2 cm, with clamps	50 L	SV50517.08
Plastic drum dolly	For 50 L, 100 L, and 200 L drums	SV50102.02
Standard Single-Use Bottle Assembly System	1 L, 2-port	SB00004-I
Standard Fluid Transfer Assembly Adaptor Set	3/8 in. ID to 1/8 in. ID capped for welding	SH31276.01
Standard Fluid Transfer Assembly Y Manifold Set	3/8 in. ID with AseptiQuik G connectors	SH31202.11
Transfer Assembly Jumper Tubing	3/8 in. ID with AseptiQuik G connectors	SH31319.11

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