gibco

AAV-MAX Helper-Free Production System, Prototype

USER GUIDE

For animal origin free, chemically defined adeno-associated viral (AAV) vector production in mammalian suspension cells

for use with:
Gibco™ Viral Production Cells 2.0, Prototype
Gibco™ Viral Production Medium, Prototype
Gibco™ AAV-MAX Transfection Kit, Prototype
Gibco™ Viral-Plex Complexation Buffer, Prototype
Gibco™ AAV-MAX Lysis Buffer, Prototype

Publication Number MAN0019921

Revision B.0

For Research Use Only. Not for use in diagnostic procedures. Product is a prototype and performance characteristics of this product have not been established.

ThermoFisher SCIENTIFIC

Manufacturer:
Life Technologies Corporation |
5781 Van Allen Way |
Carlsbad, California 92008 USA

Products:
Viral Production Cells 2.0, Prototype
AAV-MAX Transfection Kit, Prototype
AAV-MAX Lysis Buffer, Prototype

Manufacturer:
Life Technologies Corporation |
3175 Staley Road |
Grand Island, New York 14072 USA

Products:
Viral Production Medium, Prototype
Viral-Plex™ Complexation buffer, Prototype

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0019921

Revision	Date	Description
B.0	18 May 2021	Updated Harvest AAV particles section. Updated graphic In Crude AAV particle quantitation by qPCR (vg/mL) section to remove reference to benzonase.
A.0	10 February 2021	New document.

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The Gibco™ AAV-MAX Helper-Free AAV Production System, Prototype is a high-yield Adeno-Associated Virus (AAV) production system based on a clonal HEK293F-derived cell line adapted to the chemically-defined Viral Production Medium in suspension format. The AAV-MAX Helper-Free AAV Production System, Prototype includes cells, medium, transfection reagent, transfection booster, production enhancer, and lysis buffer to produce scalable, high-titer adeno-associated viral (AAV) vectors.

Contents and storage

The workflows described in this document are for use with the components in the following table.

Table 1 AAV-MAX System, Prototype Components

Contents	Cat. No.	Amount	Storage
Gibco™ Viral Production Cells 2.0[1], Prototype	A50232	1 mL (1×10^7 cells/mL)	Liquid nitrogen[2]
Gibco™ Viral Production Medium, Prototype	A49842DK	1 L	2°C to 8°C Protect from light
	A49842BA	10 L (bag)	
Gibco™ AAV-MAX Transfection Kit, Prototype: • Gibco™ AAV-MAX Transfection Reagent, Prototype • Gibco™ AAV-MAX Transfection Booster, Prototype • Gibco™ AAV-MAX Enhancer, Prototype	A50518	for 1 L culture	
	A50519	for 10 L culture	
Gibco™ Viral-Plex™ Complexation Buffer, Prototype	A49846DG	100 mL	

Table 1 AAV-MAX System, Prototype Components *(continued)*

Contents	Cat. No.	Amount	Storage
Gibco™ AAV-MAX Lysis Buffer, Prototype	A50521	100 mL	2°C to 8°C Protect from light
Gibco™ GlutaMAX™ Supplement	35050061	100 ML	Room temperature

[1] Cells are cryopreserved in 90% Viral Production Medium and 10% DMSO.

[2] Store the frozen cells in liquid nitrogen immediately upon receipt and until ready to use. **Do not store the cells at –80°C.**

System components

The following section provides descriptions of components in the AAV-MAX Production System, Prototype.

Viral Production Cells 2.0

Gibco™ Viral Production Cells 2.0 (VPCs 2.0), Prototype are a clonal derivative of the HEK293F cell line and have been adapted to suspension, high-density culture in Gibco™ Viral Production Medium. These cells can be thawed directly into Gibco™ Viral Production Medium.

Cell line characteristics:

- Transformed via culture with sheared human adenovirus 5 DNA
- Does not contain the SV40 large T antigen
- Cell doubling time of <24 hours
- Achieves maximum cell densities of >1.2 × 10^7 cells/mL in shaker flask cultures
- Maximal AAV production can be achieved between cell passages 4 to 25

Viral Production Medium

Gibco™ Viral Production Medium, Prototype is a chemically-defined, serum-free, protein-free, animal origin-free medium developed for growth and transfection of VPCs 2.0. Before use, the medium requires supplementation with 4 mM GlutaMAX™ Supplement.

AAV-MAX Transfection Reagent

Gibco™ AAV-MAX Transfection Reagent, Prototype is a chemically-defined, cationic lipid-based reagent that has been uniquely designed for high efficiency co-transfection of multiple plasmids DNA into high-density VPCs 2.0 with low toxicity.

AAV-MAX Transfection Booster

Gibco™ AAV-MAX Transfection Booster, Prototype is a chemically-defined reagent that has been uniquely designed to boost efficiency of co-transfection of multiple plasmids DNA into high-density VPCs 2.0. AAV-MAX Transfection Booster works together with AAV-MAX Transfection Reagent to deliver superior transfection efficiency for high-titer AAV vector production.

AAV-MAX Enhancer

Gibco™ AAV-MAX Enhancer, Prototype is a chemically-defined, serum-free, protein-free formulation that is designed to enhance AAV vector production in VPCs 2.0.

Viral-Plex™ Complexation Buffer

Gibco™ Viral-Plex™ Complexation Buffer, Prototype is a chemically-defined, serum-free, protein-free, phenol-red free formulation that is designed to facilitate DNA complexation with AAV-MAX Transfection Reagent during DNA transfection.

AAV-MAX Lysis Buffer

Gibco™ AAV-MAX Lysis buffer, Prototype is a ready-to-use, chemically-defined, Tween®-based cell lysis reagent for the extraction of AAV particles from producer HEK293 cells. The buffer is supplied as a 10X solution that can be directly added to HEK293 AAV production cultures to induce cell lysis.

2 Culture Viral Production Cells 2.0

Thaw and establish Viral Production Cells 2.0

Guidelines for handling cells

- **IMPORTANT!** Store the frozen cells in liquid nitrogen immediately upon receipt until ready to use. Do not store the cells at –80°C.
- Avoid subjecting cells to short-term, extreme temperature changes.
- Allow the cells to remain in liquid nitrogen for 3 to 4 days before thawing.
- For all cell manipulations, mix cells by gentle swirling and avoid vigorous shaking/pipetting.
- For routine cell culture maintenance, subculture cells every 3 to 4 days when the viable cell density reaches 4×10^6 to 6×10^6 cells/mL. Do not subculture cells that have not reached early log phase growth of $\geq 4 \times 10^6$ cells/mL.

Required materials not supplied

- 125-mL PETG Erlenmeyer Flasks (e.g., Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile, Cat. No. 4115-0125)
- Orbital shaker (e.g., MaxQ™ HP™ Tabletop Orbital Shaker, Cat. No. SHKE416HP)
- Temperature and CO_2 controlled incubator (e.g., Large-Capacity Reach-In CO_2 Incubator, Cat. No. 3950)
- Reagents and equipment to determine cell viability (e.g., hemocytometer with trypan blue or cell counter)

Thaw Viral Production Cells 2.0

1. Add 30 mL of pre-warmed Viral Production Medium supplemented with 4 mM GlutaMAX™ Supplement to a 125-mL PETG Erlenmeyer shaker flask.
2. Just before the cells are completely thawed, decontaminate the vial with 70% ethanol before opening it in a laminar flow hood.
3. Remove a vial of VPCs 2.0 from liquid nitrogen , then rapidly thaw in a 37°C water bath by swirling for 1 to 2 minutes until only a small amount of ice remains.

Note: Do not submerge the vial in the water.

4. Using a 2-mL or 5-mL pipette, transfer the entire contents of the cryovial into the shaker flask prepared in Step 1 on page 8.

5. Incubate the cells in a 37°C incubator with ≥80% relative humidity and 8% CO_2 on an orbital shaker platform.

   Note: Set the shake speed to 125±5 rpm for shakers with a 19-mm shaking diameter, 120±5 rpm for shakers with a 25-mm shaking diameter and 95±5 rpm for shakers with a 50-mm shaking diameter.

6. After 3 to 4 days post-thaw, determine the viable cell density and percent viability. Cell viability should be ≥90% with a viable cell density >1 × 10^6 viable cells/mL.

   Note: if viability is <90% on days 3 to 4 post-thaw, cells may be cultured for up to an additional 3 days in order to reach the desired viability. Cells should not be subcultured until viable cell density reaches 1 × 10^6 to 3 × 10^6 viable cells/mL.

7. For subsequent routine cell culture maintenance, subculture cells every 3 to 4 days when the viable cell density reaches 4 × 10^6 to 6 × 10^6 viable cells/mL according to Table 2.

   Note: Do not subculture cells before reaching early log phase growth of ≥4 × 10^6 cells/mL. Similarly, do not let cells overgrow above ≥6.5 × 10^6 cells/mL.

Subculture Viral Production Cells 2.0

VPCs 2.0 are capable of achieving high cell densities; therefore, it is important that cells attain a minimum density of 4 × 10^6 to 6 × 10^6 viable cells/mL at the time of subculturing.

1. Calculate viable cell density at the time of subculture.

   Note: If using a Vi-CELL™ Cell Counting instrument, see Table 2 for the recommended settings for this cell line.

2. Use the viable cell density to calculate the volume of cell suspension required to seed a new shaker flask according to the recommended seeding densities in Table 3 and the recommended culture volumes in Table 4.

Table 2 Recommended Vi-CELL™ XR Cell Counting Settings

Parameter	Value	Parameter	Value
Minimum diameter	5	Cell brightness	85
Maximum diameter	50	Cell sharpness	100
Number of images	50	Viable cell spot brightness	65
Aspirate cycles	3	Viable cell spot area	5

Table 2 Recommended Vi-CELL XR Cell Counting Settings *(continued)*

Parameter	Value	Parameter	Value
Trypan blue mixing cycles	3	Minimum circularity	0
Decluster degree	Medium		

Table 3 Recommended seeding densities for routine cell culture maintenance

Sub-culture timing	Recommended seeding density
For cells ready 3 days post-subculture	0.6×10^6 viable cells/mL
For cells ready 4 days post-subculture	0.3×10^6 viable cells/mL

Table 4 Recommended volumes for routine cell culture maintenance

Flask size	Culture volume[1]	Shake speed
125 mL	30 mL	125 ± 5 rpm (19-mm orbital diameter) 120 ± 5 rpm (25-mm orbital diameter) 95 ± 5 rpm (50-mm orbital diameter)
250 mL	60 mL	
500 mL	120 mL	
1 L	240 mL	
2 L	480 mL	
2.8 L	700 mL	90± 5 rpm (19-mm orbital diameter) 85± 5 rpm (25-mm orbital diameter) 80± 5 rpm (50-mm orbital diameter)

[1] If using volumes outside of the recommended range, it is critical to ensure that all cell growth (i.e., doubling times), health (i.e., cell diameter, viability), and expression levels remain consistent with control conditions. Cell performance is decreased if cell health is compromised.

3. Transfer the appropriate number of cells to fresh, pre-warmed Viral Production Medium supplemented with 4 mM GlutaMAX™ Supplement in a shaker flask.

4. Incubate the flasks in a 37°C incubator with ≥80% relative humidity and 8% CO_2 on an orbital shaker platform until the cultures reach a density of 4×10^6 to 6×10^6 viable cells/mL.

Note: Cells that are subcultured at densities outside of this early log-phase growth window may show longer doubling times and lower titers over time. Modify the initial seeding density to attain the target cell density of 4×10^6 to 6×10^6 viable cells/mL at the time of subculturing.

5. Repeat step 1 to step 3 to maintain or expand cells for transfection.

Cryopreserve Viral Production Cells 2.0

VPCs 2.0 can be frozen directly in Viral Production Medium supplemented with 4 mM GlutaMAX™ Supplement with 10% DMSO. Alternatively, conditioned cryopreservation medium consisting of 45% fresh Viral Production Medium supplemented with 4 mM GlutaMAX™ Supplement, 45% conditioned medium and 10% DMSO can be used.

1. Prepare the freezing medium with 90% Viral Production Medium supplemented with 4 mM GlutaMAX™ Supplement + 10% DMSO and place on ice until cells are ready to use.
2. Determine the viable cell density and calculate the volume of cell culture needed for cryopreservation.
3. Centrifuge the calculated amount of cell volume at 300 × *g* for 5 minutes. Discard the supernatant without disturbing cell pellet.
4. Add a smaller volume (10% of final banking volume) of cold freezing medium to the pellet, then gently resuspend the cell pellet by pipetting. Once resuspended, further dilute the cells to a final density of 1×10^7 viable cells/mL in freezing medium.
5. Freeze the cells in an automated or manual controlled-rate freezing apparatus following standard procedures. For manual processes, freeze the cells at -80°C for overnight prior to transfer the frozen cells to liquid nitrogen. For ideal cryopreservation, the rate of temperature decrease should be 1°C per minute.
6. Transfer frozen the vials to liquid nitrogen for long-term storage.

3 Transfect Viral Production Cells 2.0

Transfection guidelines

- Allow freshly thawed cells to recover in culture for three or more passages post-thaw and before transfection.
- During all cell manipulations, mix the cells by gentle swirling; avoid vigorous mixing/pipetting. Cell health is critical to maximal performance.
- Gently invert the AAV-MAX Transfection Reagent 4 to 5 times before use to ensure thorough mixing.
- Complexation of plasmid DNA and AAV-MAX Transfection Reagent takes place at room temperature.
- See Table 5 for transfection at various scales.

Equipment guidelines

- For optimal performance, it is important to follow the recommended shaking diameter, shaking speed, flask size/type, and volume of culture to be transfected provided in this protocol.
- Humidified incubators (≥80% relative humidity) are recommended to reduce evaporation during AAV production runs. When using multi-well plates, use high-humidity settings if available, as evaporation can introduce variation and impact results.
- Ensure equipment is calibrated for temperature. In some instances, the total heat from the incubator and the shaker can cause cell culture temperatures to exceed the recommended ranges and lead to decreased cell growth, clumping or cell death. In such instances, reduce the temperature setting of the incubator to compensate for heat generated by the shaker.
- Ensure that equipment is calibrated for CO_2. Levels of CO_2 should not exceed 8%.

Required materials

- Viral Production Cells 2.0 cultured in Viral Production Medium supplemented with 4 mM GlutaMAX™ Supplement
- User-specific plasmids: Transfer plasmid (containing the gene of interest), Rep/Cap plasmid, Helper plasmid.
- AAV-MAX Transfection Kit
- Viral-Plex™ Complexation Buffer
- Viral Production Medium supplemented with 4 mM GlutaMAX™ Supplement, pre-warmed to 37°C
- Disposable, sterile PETG Erlenmeyer flasks

- Orbital shaker in a 37°C incubator with ≥80% relative humidity and 8% CO_2
- Reagents and equipment to determine viable cell density and percent viability

Optimized transfection conditions

Condition	Amount
Viral-Plex™ Complexation Buffer	100 µL per mL of culture volume to transfect
Plasmid DNA[1]	1.5 µg per mL total plasmid DNA of culture volume to transfect (i.e. Transfer plasmid + Rep/Cap plasmid + Helper plasmid)
AAV-MAX Transfection Reagent	6 µL per mL of culture volume to transfect
AAV-MAX Transfection Booster	3 µL per mL of culture volume to transfect
AAV-MAX Enhancer	10 µL per mL of culture volume to transfect
AAV-MAX Lysis Buffer	100 µL per mL of culture volume to transfect
Viral Production Cells 2.0	3 x 10^6 cells/mL density at transfection

[1] Optimizing the molar ratio of plasmids based on plasmid size is highly recommended. An example calculation for appropriate amounts of the 3 plasmids based on plasmid size is depicted in Table 6.

Table 5 Recommended reagent volumes for transfection at various scales

Vessel type	125 mL flask	250 mL flask	1 L flask	2 L flask	2.8 L flask
Number of cells required	90 × 10^6	180 × 10^6	720 × 10^6	1,440 × 10^6	3,000 × 10^6
Culture volume to transfect	30 mL	60 mL	240 mL	480 mL	1,000 mL
Shake speed [1]	125 ± 5 rpm (19 mm orbital diameter) 120 ± 5 rpm (25 mm orbital diameter) 95 ± 5 rpm (55 mm orbital diameter)				90 ± 5 rpm 90 ± 5 rpm 55 ± 5 rpm
Amount of plasmid DNA	1.5 µg total plasmid DNA per mL of culture to transfect				
Volume of plasmid DNA [2]	45 µL	90 µL	360 µL	720 µL	1.5 mL
Viral-Plex™ Complexation Buffer to dilute plasmid DNA [3]	3 mL	6 mL	24 mL	48 mL	100 mL
AAV-MAX Transfection Reagent	180 µL	360 µL	1.44 mL	2.88 mL	6 mL
AAV-MAX Transfection Booster	90 µL	180 µL	720 µL	1.44 mL	3 mL

Table 5 Recommended reagent volumes for transfection at various scales *(continued)*

Vessel type	125 mL flask	250 mL flask	1 L flask	2 L flask	2.8 L flask
AAV-MAX Enhancer	300 µL	600 µL	2.4 mL	4.8 mL	10 mL
Final culture volume	~34 mL	~68 mL	~272 mL	~544 mL	~1,120 mL

[1] Recommended shake speed ranges: optimal shake speed should be determined empirically based on the specific laboratory equipment used.
[2] Assuming a plasmid DNA concentration of 1 mg/mL and a final concentration of 1.5 µg/mL of plasmid DNA.
[3] Volume used to dilute plasmid DNA.

Transfection procedure

Refer to Table 5 for suggested volumes for transfection at various scales.

Subculture and expand cells until the cells reach a density of approximately 4×10^6 to 6×10^6 viable cells/mL.

Day 0: Prepare and transfect cells

1. On the day of transfection (Day 0), determine viable cell density and percent viability. Cells should have reached a density of approximately 4.5×10^6 to 6.0×10^6 viable cells/mL. Viability should be ≥95% to proceed with transfection.

2. Dilute the cells from step 1 to a final density of 3×10^6 viable cells/mL with fresh Viral Production Medium supplemented with 4 mM GlutaMAX™ Supplement.

3. Immediately add AAV-MAX Enhancer (i.e. add 300 µL of Enhancer to transfect 30 mL of cells). Swirl the flask(s) gently to mix the cells. Incubate the cells on an orbital shaker in a 37°C incubator with a humidified atmosphere of 8% CO_2 until the DNA/Transfection complexation process is completed (step 5 to step 7). For suggested shake speeds, see Table 4.

4. Prepare Transfer plasmid DNA, Rep/Cap plasmid DNA and Helper plasmid DNA.
   a. Use 1.5 µg /mL of total plasmid DNA to the culture volume to be transfected (See Table 5 for the recommended volumes at various scales).

   Note: Plasmid DNA molar ratio optimization based on plasmid size is highly recommended. See Appendix A, “Additional guidelines” for example of ratio optimization of 3 plasmids DNA.

5. Prepare Transfection complexes as described (See Table 5 for the recommended volumes at various scales).
   a. Gently invert the AAV-MAX Transfection Reagent bottle 4 to 5 times to mix.

   b. Dilute total plasmid DNA from step 4 (e.g., 45 µg total DNA to transfect a 30 mL culture) with Viral-Plex™ Complexation Buffer to a final volume of 10% of the culture volume to be transfected (e.g., 3 mL Viral-Plex™ Complexation Buffer to transfect a 30 mL culture).

   c. Mix by swirling gently and incubate the diluted DNA at room temperature for 10 min.

d. To a new tube, add neat AAV-MAX Transfection Booster at 3 µL per mL of culture to be transfected (e.g., 90 µL Booster to transfect a 30 mL culture) followed by neat AAV-MAX Transfection Reagent at 6 µL per mL of culture to be transfected (e.g., 180 µL AAV-MAX Transfection Reagent to transfect a 30 mL culture).

e. Mix by gentle pipetting (do not vortex) and incubate at room temperature for 10 min.

f. Add the pre-mixed AAV-MAX Transfection Booster and AAV-MAX Transfection Reagent (e.g., 90 µL AAV-MAX Booster + 180 µL AAV-MAX Reagent) from substep 5e to the diluted plasmid DNA fromsubstep 5c. Mix by swirling, inversion or gentle pipetting 2 to 3 times (do not vortex).

6. Incubate plasmid DNA/AAV-MAX Booster/ AAV-MAX Reagent complexes from substep 5f at room temperature for 20 to 25 minutes, then gently transfer the solution to the shaker flask(s) prepared in step 3.

7. Incubate the cells on an orbital shaker in a 37°C incubator with a humidified atmosphere of 8% CO_2 for approximately 72 hours. For suggested shake speeds, see Table 4.

Harvest AAV particles

Handling of AAV particles must be performed as per institutional guidelines. All materials that come into contact with AAV solution should be appropriately disinfected prior to disposal.

The AAV-MAX Lysis buffer is a 10X formulation that can be added directly to the cell culture to induce lysis.

Harvest AAV particles 70 to 72 hours post-transfection.

1. Add AAV-MAX Lysis Buffer directly to the culture flask at a 1:10 dilution (e.g., 3.3 mL of Lysis buffer to a 30 mL culture volume) and swirl the flask to evenly distribute the lysis buffer.

Note: If performing qPCR measurement of titers, remove 5 mL from the culture flask after performing step 1 and perform lysis by incubating at 37°C for 1 hour at 250 rpm on an orbital shaker. Proceed to step 3.

Note: If proceeding to a downstream workflow, add $MgCl_2$ (final concentration: 2 mM) and Benzonase (final concentration: 90 U/mL) to the remaining culture in the flask from step 1 ,then proceed to step 2.

2. Incubate the flask at 37°C for at least 2 hours on an orbital shaker (for suggested shake speeds, see Table 4).

Note: Once cells are lysed, the culture will appear to contain visible cell debris.

Note: Lysis incubation times can vary based on AAV serotype and production scale. Therefore, it is recommended to optimize lysis conditions for your experiments prior to moving to large scale.

3. Transfer the cell lysate to an appropriate flask or tube, then centrifuge at 4°C at 13,000 × *g* for 10 min for smaller volumes (< 2 mL) or at 4,500 × *g* for 30 minutes for larger scale of production.

4. To harvest crude AAV particles, transfer the supernatant to an appropriate storage container.

5. For qPCR titer measurement, transfer 50 to 100 µL of lysate to a 96-well plate.

Note: We recommend pairing multiple replicates for each sample to account for qPCR assay variation.

6. Harvested crude AAV particles can be stored at -80°C for long term storage.

Note: To avoid repeated freeze/thaw cycles, virus aliquoting is highly recommended. To thaw AAV samples, bring the tube to room temperature, then mix the AAV sample by gentle pipetting or inverting. Do not vortex or avoid mixing vigorously.

Note: Crude AAV vectors can temporarily be stored at 4°C for short duration (i.e. overnight). If samples are stored for extended periods of time at 4°C, precipitation can occur. This is dependent on AAV serotype and/or production scale. If precipitation is observed, reclarify the samples prior to proceeding to the next step.

Note: To disinfect AAV properly, prepare a 10% bleach solution, then disinfect used pipette tips, serological pipettes and culture flasks before disposal. To discard remaining AAV samples, add the 10% bleach solution directly to the AAV solution, then incubate for a minimum of 30 minutes before disposal.

A Additional guidelines

Molar ratio optimization of plasmids

To achieve the highest transfection efficiency for AAV production, molar ratio optimization of the three plasmids (Transfer plasmid, Rep/Cap plasmid and Helper plasmid) based on plasmid size is highly recommended. For AAV production using the AAV-MAX system, 1.5 mg of total DNA is required for 1L of AAV production. Based on the total number of base pairs (bp) of all 3 plasmids, you can determine total µg/bp that is needed to use for each plasmid. An example calculation for appropriate amounts of the 3 plasmids based on plasmid size is depicted in Table 6.

Table 6 Example calculation of plasmid DNA amounts based on plasmid size

Plasmid	Plasmid Size (bp)	Molar ratio	Plasmid Size considering molar ratio (bp)
Transfer Plasmid	5,400	1	5,400
Rep/Cap Plasmid	7,330	2	14,660
Helper Plasmid	11,635	0.5	5,818
Total bp	24,365		25,878

1500 µg/25878 bp = 0.06 µg/bp. The following table summarizes the amount needed for each plasmid:

Plasmid	Total DNA required
Transfer Plasmid	0.06 µg/bp × 5,400 bp = ~325 µg
Rep/Cap Plasmid	0.06 µg/bp × 14,660 bp = ~880 µg
pHelper	0.06 µg/bp × 5,818 bp = ~350 µg

Crude AAV particle quantitation by qPCR (vg/mL)

For AAV production using triple transfection, Transfer plasmid, Rep/Cap plasmid and Helper plasmid are co-transfected into producer cells (Figure 1, left). The subsequently produced AAV particles contain a mixture of full, partial and empty capsids, meaning that there will be residual single strand DNA (ssDNA) that have not been packaged into the AAV particles. In order to measure an accurate AAV titer, residual plasmid and excess ssDNA need to be removed through DNase treatment (Figure 1, right), followed by treatment with proteinase K to digest capsid proteins and release ssDNA for qPCR quantification. Once enzyme digestion is completed, qPCR assay is prepared using primers and probes specific to the Gene of Interest (GOI). For the most accurate titer measurement, it is recommended that qPCR conditions, including primers and probes design based on serotypes and GOI, are optimized. All materials needed for AAV quantification by qPCR are available through thermofisher.com.

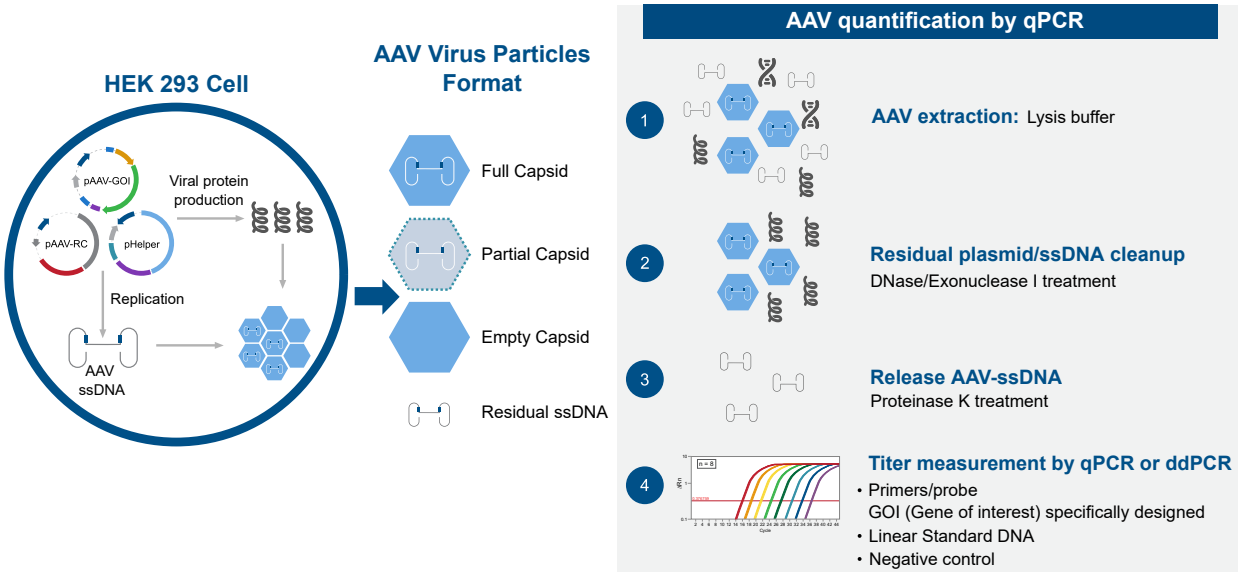

Figure 1 Illustration of AAV production using triple transfection and qPCR titer measurement procedure

Item	Cat. No.
UltraPure™ DNase/RNase Free Water	10977015
DNase I	18047019
10X DNase I Reaction Buffer	AM8170G
Proteinase K	AM2548
Platinum™ Quantitative PCR SuperMix-UDG w/ROX™	11743500

B Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.