Description

The AAV qPCR Titration Kit is a kit designed to measure AAV (adeno-associated virus) titers of particles with AAV2 ITR (inverted terminal repeats) by qPCR (quantitative polymerase chain-reaction). It offers a streamlined, one-step process without needing digestion steps. Engineered for high sensitivity and specificity, it minimizes non-specific background, enhancing performance over comparable kits. The kit contains enough reagents for 100 reactions. It includes a separate ROX (carboxyrhodamine), a passive fluorescence reference dye that allows for signal normalization, and an AAV reference material as qPCR standard for precise quantification.

This product has been validated across multiple AAV samples with varying serotypes, purification status, and encapsulated transgenes, consistently demonstrating high levels of accuracy, precision, and robustness.

Background

Recombinant adeno-associated virus (rAAV) is a widely used vector in gene therapy applications. rAAVs exist in the nucleus as episomes, and are unable to integrate into the cellular genome. While this brings safety benefits, as there is no risk of random integration and activation of oncogenes, the cell division rate dictates the timeframe for transgene loss. Their application is thus particularly useful for the delivery of transgenes to post-mitotic tissues like the brain, retinal, liver, skeletal muscle, and the heart. The payload carried by AAVs is somewhat smaller than other viral tools use for transduction, usually smaller than 5 kb. AAV titers can be measured as a physical titer, infectious titer, or ratio of full to empty capsids. Accurately determining the genome titer is essential not only for setting clinical doses but also as a foundational step in various analytical assays for characterizing AAV products. While AAV genome titers, physical titers, are typically measured by qPCR (quantitative polymerase chain reaction), precision has remained a challenge despite numerous refinements to the process. Typically, standard curves are done with plasmid DNA, which does not provide a direct correlation with material of interest. The use of an assay that relies on digestion-free method, includes an AAV standard and reference material, is well-suited for adoption in gene therapy research and can be further automated for high-throughput applications.

Application

- AAV with AAV2 ITR qPCR titration

Supplied Materials

Catalog #	Name	Amount	Storage
	SYBR Green™ 2x qPCR MasterMix	1000 µl	-20°C
	Primer Mix	200 µl	-20°C
	Dilution Buffer	5000 µl	-20°C
	DNase I Reaction Mix	1000 µl	-20°C
	AAV Reference	30 µl	-20°C
	ROX Reference Dye	15 µl	-20°C
	Nuclease-Free H_2O	2 x 1000 µl	-20°C

Materials Required but Not Supplied

- Adjustable micropipettor and sterile tips
- Rotating or rocker platform
- qPCR instrument
- PCR tubes or plate
- AAV sample of interest

Storage Conditions

Components are shipped in dry ice and stored at -20°C freezer for long term storage. The components maintain their stability and performance up to 5 freeze-thaw cycles.

BioSafety

This product is for research purposes only and not for human or therapeutic use. Overall, this product should be considered hazardous and harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly. Recombinant AAV is inherently replication-deficient and not known to cause any human diseases. Additionally, following transduction, AAV vectors exist episomally and do not integrate into or disrupt the host cell's genome. AAV requires the use of a Biosafety Level 1 facility. BPS Bioscience recommends following all local, federal, state, and institutional regulations and using all appropriate safety precautions.

Assay Protocol

- This kit targets the AAV2 ITR sequence to quantify viral genome levels, making it compatible with any serotype as long as the vector genome's ITR region originates from AAV2. Before use, please verify that the ITR domain of your AAV vector is indeed derived from AAV2.
- Traditionally, AAV particles are extracted from AAV-producing cells using freeze-thaw cycles or sonication, though these techniques can be time-intensive and require specialized equipment. AAV ONE-Extract™ Solution (#78585, sold separately) provide a quick and efficient alternative for extracting AAV particles, reducing protein and nucleic acid contaminants in the process.
- We recommend all reactions are set-up on ice in duplicate.
- The assay should include "AAV Reference" (Positive Control) and "NTC" (No Template Control) conditions.
- The suggested quantity of ROX Reference Dye to include in the Master Mix differs depending on the type of qPCR instrument:
    - For equipment that does not require ROX: no dye is needed.
    - For low ROX instruments: use 1 µl of ROX Reference Dye per 1000 µl of Master Mix.
    - For high ROX instruments: add 10 µl of ROX Reference Dye per 1000 µl of Master Mix.

DNase I Treatment

1. Mix 2 µl of the AAV sample with 18 µl of DNase I Reaction Mix.
2. Mix 2 µl of the AAV Reference with 18 µl of DNase I Reaction Mix.
3. Incubate at 37°C for 30 minutes to remove free gDNA, plasmid DNA, and unpackaged viral DNA from host cells.
4. Dilute DNase I-treated AAV samples according to the dilution scheme shown in the table below:

Dilution Series	Volume of AAV Sample (µl)	Volume of Dilution Buffer (µl)	Dilution factor	Total dilution
Dilution 1 (DNase step)	2 µl	18 µl	10 X	100 X
Dilution 2	2 µl of Dilution 1	18 µl	10 X	1000 X
Dilution 3	2 µl of Dilution 2	18 µl	10 X	10000 X
Dilution 4	2 µl of Dilution 3	18 µl	10 X	100000 X
Dilution 5	2 µl of Dilution 4	18 µl	10 X	1000000 X

Note: Dilutions 2–4 are suitable for most samples. If sample is expected to have a titer >1 x 10^{13} VG/ml, use dilutions 3-5.

5. Prepare a serial dilution of AAV Reference (1 x 10^9 VG/µl stock), as described in the table below:

Dilution Series	Volume of AAV Reference stock or previous dilution (µl)	Volume of Dilution Buffer (µl)	VG/µl
Dilution 1 (DNase step)	From DNase step	0	1 x 10^8
Dilution 2	2 µl of Dilution 1	18 µl	1 x 10^7
Dilution 3	2 µl of Dilution 2	18 µl	1 x 10^6
Dilution 4	2 µl of Dilution 3	18 µl	1 x 10^5
Dilution 5	2 µl of Dilution 4	18 µl	1 x 10^4
Dilution 6	2 µl of Dilution 5	18 µl	1 x 10^3

qPCR Set-Up

1. Prepare the following reactions on ice in duplicate, as described in the table below:

Component	AAV Sample	AAV Reference	NTC Sample
SYBR Green™ 2X qPCR MasterMix	10 µl	10 µl	10 µl
Primer Mix	2 µl	2 µl	2 µl
Diluted AAV Sample	2 µl	-	-
Diluted AAV Reference	-	2 µl	-
NTC	-	-	2 µl
Nuclease-Free H_2O	6 µl	6 µl	6 µl
Total	**20 µl**	**20 µl**	**20 µl**

2. Setup the qPCR cycling conditions as follows:

Step	Temperature (°C)	Duration	Cycles
Initial Denaturation	98°C	3 minutes	1
Denaturation	98°C	15 seconds	35
Annealing/Extension	65°C	20 seconds	

Melt Curve Analysis

1. Plot Ct value (Y-axis, linear scale) vs. Virus titer (X-axis, logarithmic scale) for the "AAV Reference" control.

   Apply logarithmic regression to determine the unknown virus sample titer using the equation *y = mx + b* from the trendline equation:
   Virus titer (VG/ml) = $e^{(Ct - b)/m}$, where **m** is the slope of the line and **b** is the y-intercept.

   For example: if the trendline equation is y = -1.298 ln(x) + 40.978; **Ct** of unknown sample = 17.08, the viral titer is (VG/ml) = $e^{(17.08 - 40.978)/-1.298}$ = 9.91 x 10^7 VG/ml.

   Note: The R^2 value should be > 0.95.

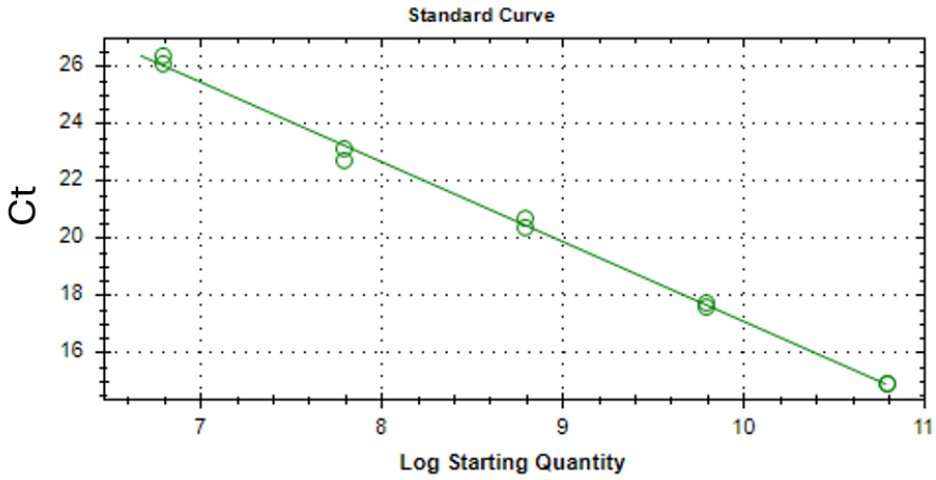

Figure 1: Standard curve generated with the AAV Reference.

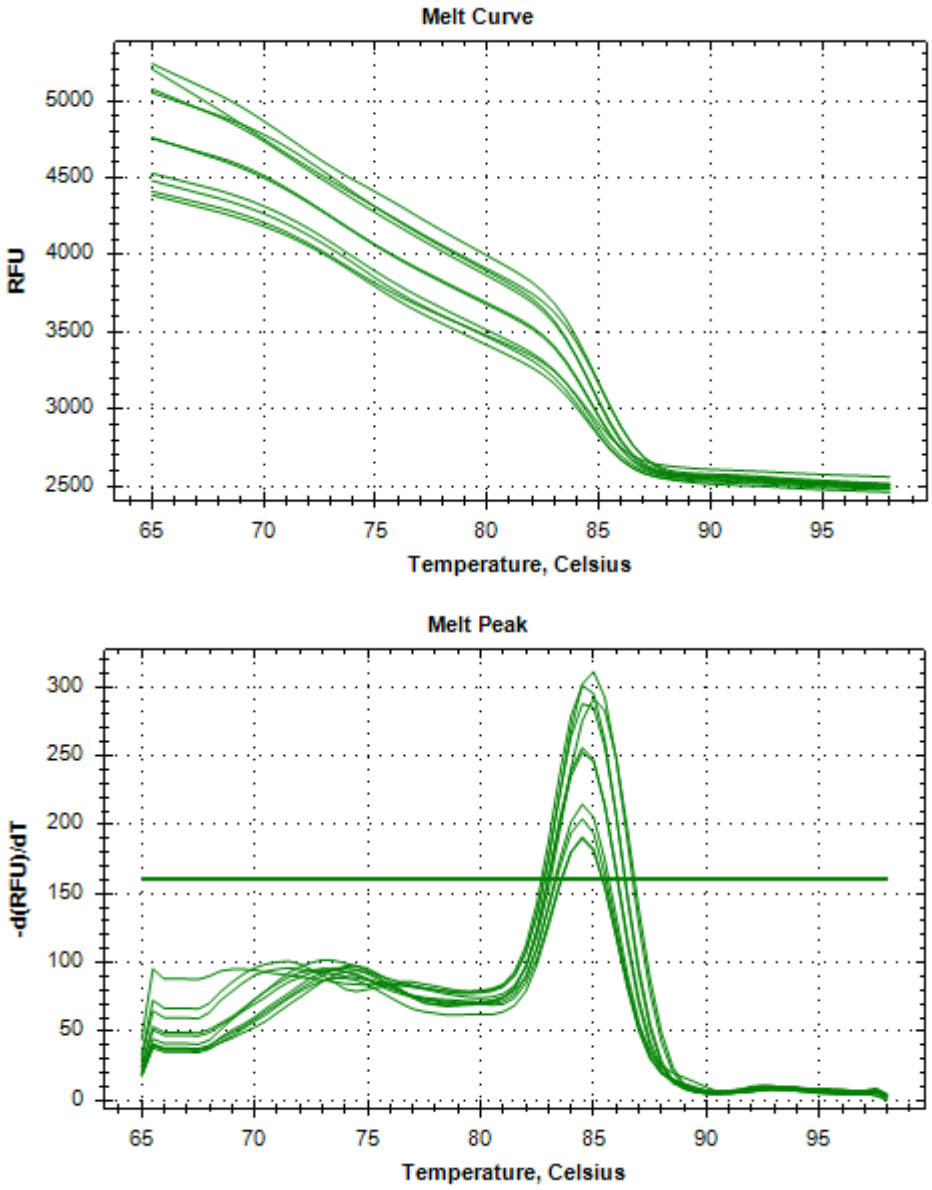

Figure 2. Melt curve analysis of qPCR products.
The melt curve was generated by gradually increasing the temperature from 65 °C to 98 °C while monitoring fluorescence (RFU). A sharp decrease in fluorescence between 80 °C and 90 °C indicates the melting temperature (Tm) of the specific PCR products. The presence of a single melting transition suggests specificity of amplification with minimal primer-dimer or nonspecific products.

Validation Data

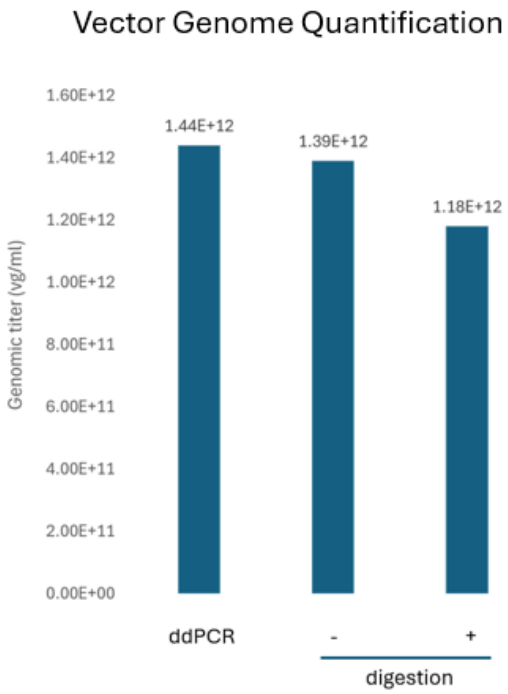

Figure 3: AAV8 titer measurements using three methods: ddPCR, digestion-free qPCR quantification, and Proteinase K digestion qPCR.
Digital droplet PCR (ddPCR), digestion-free qPCR quantification, and Proteinase K digestion qPCR method were conducted. The titer values obtained by ddPCR and the digestion-free method were closely matched, indicating higher accuracy compared to the Proteinase K digestion approach.

Data are representative.

Troubleshooting Guide
Visit bpsbioscience.com/aav-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
AAV ONE-Extract™ Solution	78585	15-150 preps
AAV2 ZsGreen	78444	50 µl x 2
AAV2 SaCas9	78480	50 µl x 2
AAV2 Luciferase	78453	50 µl x 2
AAV2 Luciferase-eGFP	78462	50 µl x 2
AAV2 Luciferase-mCherry	78471	50 µl x 2

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