AAV qPCR *fast* Titer Kit

Catalog #: TR30042

For Research Use

Description

The AAV qPCR Fast Titer Kit can be used to quantitate AAV titers through the measurement of AAV genome copies using SYBR green I chimeric fluorescence technology in a highly specific, sensitive and accurate way. The kit contains Specific ROX Reference Dye, which is suitable for most qPCR instruments and has a quick lysis step. The AAV qPCR Fast Titer Kit provides a convenient method to quantitate AAV amount in less than 2 hours.

Materials Supplied

The reagents supplied in this pack are for Research Use Only.

Description	Quantity
2x Universal SYBR qPCR Master Mix*	1 ml
Primers Mix**	100 µl
Accelerator Buffer	1.5 ml
Standard (2 x 10^9 GC/ml)	100 µl
UltraPure™ DNase/RNase-Free Distilled Water	1.5 ml

* It contains dNTP, Mg^{2+}, Taq Pro DNA Polymerase, SYBR Green I and Specific ROX Reference Dye, etc.

**The primers were designed based on ITR sequence.

Storage and Stability

Upon receipt, store the kit at -20°C protected from the light. The kit should not be used beyond the expiration date.

Assay Procedure:

1. Optional Digestion

Recommended for crude viral samples.

Digest un-encapsidated DNA using DNase I

Preparation for the Assay

Prepare a reaction setup (shown in table 1) and incubate for 30 min at 37°C, then cool to 4°C.

Component	Volume/Reaction (µl)
10x DNase I Reaction Buffer	5
DNase I (2U/µl)*	5
DNase/RNase-Free Distilled Water	35
AAV sample	5
Total volume	**50**

Table 1. Reaction setup for digestion

* DNase I (RNase-free) (10x DNase I Reaction Buffer is included with the DNase I; New England Biolabs [NEB] Ltd., Cat. No. M0303)

2. Lyse capsid:

Add 5 µl of AAV sample into 45 µl Accelerator Buffer, mix, and incubate at room temperature for 5 min.

Note: The AAV sample has been diluted 1/10 (only lyse capsid) or 1/100 (DNase I treatment + Capsid lysis), thus take this dilution factor into consideration when calculating the final titer

3. Dilute AAV samples (Dilute the AAV to 10^8 GC/ml range):

For initial experiments on purified AAV of unknown concentration, we recommend preparing tenfold serial dilutions and use 1/1,000 to 1/100,000 diluted viral samples for measurements. For example, this can be done by placing 10uL of the lysis sample mixture with 90uL of DI water, making a 1:100 dilution. Thoroughly mix before proceeding to the next dilution, then placing 10uL of the 1:100 dilution into another 90uL of DI water to make a 1:1000 dilution…, then placing 10uL of the 1:10,000 dilution into another 90uL of DI water making a 1:100,000 dilution.

4. Dilute standard:

Prepare six tenfold serial dilutions from the stock standard that is at concentration of 2.00 x 10^9 GC/mL. For example, mix 10uL of stock solution into 90 uL of DI water to generate a 2.00 x 10^8 GC/mL solution, followed by 10uL of the 2.00 x 10^8 GC/mL solution into 90uL of DI water to generate a 2.00 x 10^7 GC/mL solution, and so on. Repeat for all other dilutions.

Table 2. Standard dilution

Standard	Dilution	Stock Concentration	uL, Used in 20 uL qPCR Reaction	Concentration in qPCR Reaction
1 (stock standard)		2.00 x10^9 GC/mL	5	1.00 x10^{10} GC/mL
2	1:10	2.00 x10^8 GC/mL	5	1.00 x10^9 GC/mL
3	1:10	2.00 x10^7 GC/mL	5	1.00 x10^8 GC/mL
4	1:10	2.00 x10^6 GC/mL	5	1.00 x10^7 GC/mL
5	1:10	2.00 x10^5 GC/mL	5	1.00 x10^6 GC/mL
6	1:10	2.00 x10^4 GC/mL	5	1.00 x10^5 GC/mL
7	1:10	2.00 x10^3 GC/mL	5	1.00 x10^4 GC/mL
8 (blank)		0	5	0

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5. Prepare qPCR reactions:

qPCR reaction setup is shown in Table 3. All steps are recommended to be done on ice.

Table 3. Reaction setups for qPCR

Component	Volume/Reaction (**µl**)
2x qPCR Master Mix	10
Primers Mix	1
Nuclease-free H2O	4
Sample, NTC*, or Standards	5

*NTC: Negative control

6. Prepare qPCR Amplification:

Using the qPCR cycling conditions in Table 4 for qPCR amplification.

Table 4. qPCR cycling conditions

Cycling Step	Cycle(s)	Temperature	Time
Enzyme activation	1	95°C	30 sec-3 min[1]
Denaturation		95°C	3-10 sec[2]
Annealing/extension	40	60°C	10-30 sec[3]

[1]. For standard program, select 30 sec, but 3 min can be selected to improve the effect.
[2].For standard program, select 10 sec, and 3 sec can be selected for fast program.
[3]. Select 30 sec for standard program; Fast program: for amplicons within 200 bp, the shortest extension time can be set to 10 sec.

7. Analyze Data:

This is data analysis to obtain titer readings from cycle thresholds (C_t value) to GC/mL (genome copies per milliliter).

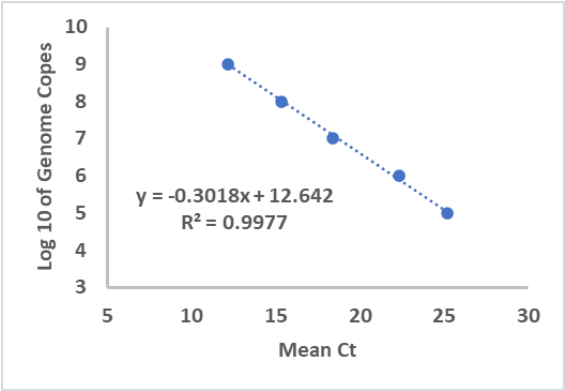
1) Export the Ct values to an Excel spreadsheet.
2) For the standard curve, plot $\log_{10}$ of the standard titer versus the mean C_t. Generate a logarithmic regression using the five standard titers, add a trendline and display the equation (y = ax + b). The R^2 value should be > 0.95 to justify the proper assay setup.
3) Determine the tier of the unknown samples using the C_t values and the equation generated from the trendline (y = ax + b).
4) Calculate AAV titers from the qPCR data.

AAV titer (GC/mL) = 10^(y) × sample dilution factor/Volume of sample used

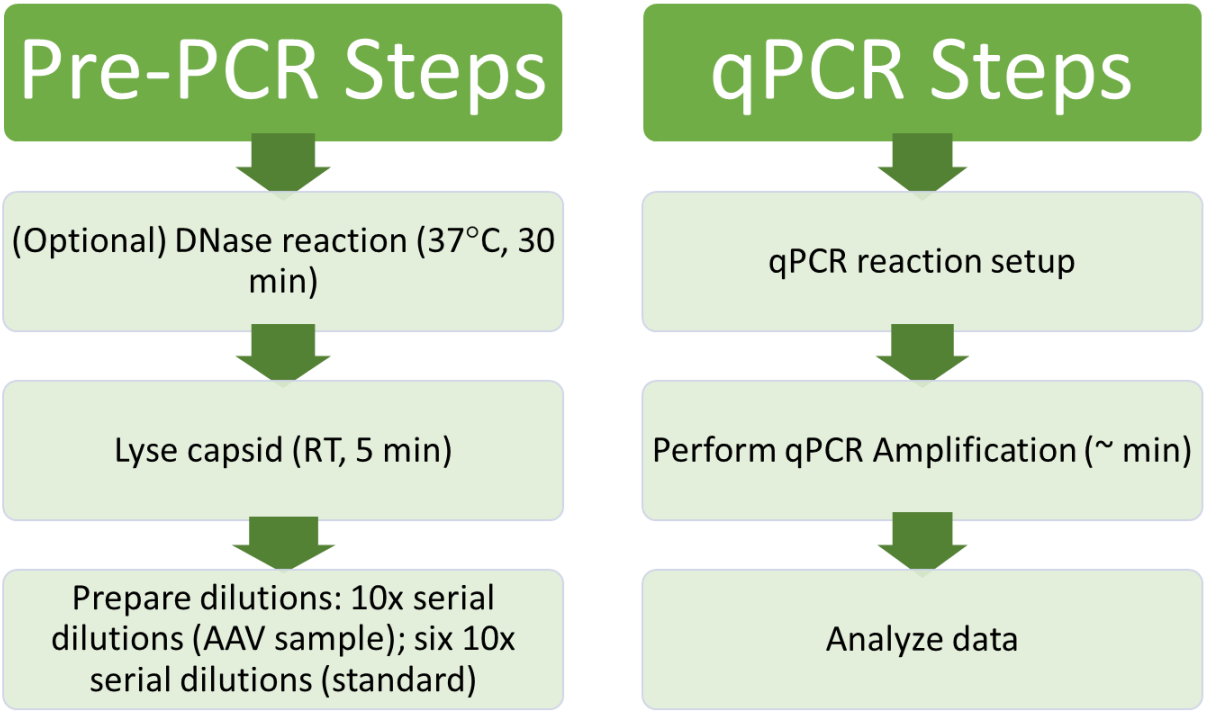
For example:

Table 5. To obtain standard equation

Standard Genome Copies	Log10 of Genome Copies	Average CT
1.00E+09	9	12.17459
1.00E+08	8	15.34979
1.00E+07	7	18.37857
1.00E+06	6	22.36145
1.00E+05	5	25.1954



Assay Flowchart



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