Universal AAV qPCR FAST Titer (100 Tests) Catalog #: TBS4054-100

Product Description

TribioScience's AAV qPCR FAST Titer Kit incorporates TribioScience FAST technology paradigm and Probe Taqman qPCR technology for an accurate, one-step, and time-saving qPCR titration of AAV serotypes. The probe is labelled with Fluorescence FAM. The entire procedure can be completed within 2-3 hours. With its time-saving 1-minute enzyme activation technology, specificity, accuracy and sensitivity, the performance of TribioScience's AAV qPCR FAST Titer Kit is unmatched by similar kits currently on the market. The Kit performances are listed Fig.1.

Kit Content for 100RXN

Component	Volume	Part Number
FAST Lysis Buffer	0.8 mL	AAV001
AAV Fast qPCR mix	1.0 mL	AAV002
AAV Primer-Probe Mix	0.6 mL	AAV003
AAV Control DNA	30 mL	AAV004
DNase I Reaction Mix	1.0 mL	AAV005
Nuclease Free Water	1.5 mL	AAV006

Note: The probe is labelled with Fam.

Storage and Shelf-life

Store at -25ºC to -15ºC immediately upon arrival. Minimize the number of freeze-thaw cycles to ensure superior performance. The Kit is stable for one (1) year from the date of arrival.

Protocol

1. **Viral Sample Preparation**

A. **Purified viral particles:** Bring viral particles to 10^8 GC/ml with PBS (Note: GC is gene copy).

B. **Virus producing cell cultures**: centrifuge cell culture medium at 2000g for 5 minutes to sediment suspended cells and cell debris. Use supernatant for the next step.

2. **(Optional) DNase I Treatment recommended for crude viral samples:** Add 2 mL of AAV sample into 18 mL of DNase I Reaction Mix. Incubate samples at 37℃ for15 minutes to digest free gDNA, plasmid DNA and unpackaged viral DNA derived from host cells. Incubate at 95℃ for 10minutes to inactivate DNase I

3. **Viral Lysis**
Add 2 mL of the sample preparation from Step 1 or Step 2 to 18 mL of FAST Lysis Buffer (1:10 dilution). Pipette gently up and down to mix and incubate at room temperature (23ºC) for 3 minutes. Use the lysed sample for the reaction set up in Step5. *Note: the viral sample has been diluted 1/10, thus take this dilution factor into consideration when calculating the final titer.*

4. **Standard Control DNA Preparation**: The AAV Control DAN is $2x10^{10}$ GC/mL in the kit. Perform 6 serial dilutions of the Standard Control DNA at 10-fold manner by diluting 2 mL Standard DNA into next 18 mL Nuclease-Free water in each concentration as Fig. 1. Dilutions 1/10 to 1/1000000 will be used for generating the standard curve. The GC is in the table1:

Fig.1: DNA Standard Preparation

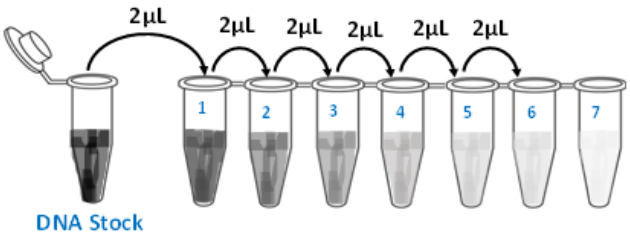

Table 1: AAV DNA Standard Concentration

Dilution	Virus Titer (GC/ml)
1/10	$2x10^9$
1/100	$2x10^8$
1/1000	$2x10^7$
1/10000	$2x10^6$
1/100000	$2x10^5$
1/1000000	$2x10^4$
Negative	0

5. **qPCR Preparation:** All reactions are set up on ice in duplications. The reaction volume is 25 mL.

Table 2: AAV qPCR Reaction Mix Preparation

Component	Volume (mL)
Fast qPCR mix	8.0
Primer-Probe mix	4.0
DNA Sample or Standard, NTC	5.0
Nuclease Free Water	8.0
Final Volume	25

6. **qPCR Running Parameters:** Program the qPCR instrument as Table 3, and Color Channel is FAM:

Table 3: qPCR Reaction Parameters

Reaction Step	Temperature (℃)	Time	Cycle(s)
Enzyme Activation	95	60S	1
Denaturation	95	15S	40
Priming/Extension	60	60S	

7. **Titer Calculation**
Plot Ct value (Y-axis, Linear scale) vs. Virus titer (X-axis, Logarithmic scale). Generate a logarithmic regression using the four (4) Standard Control DNA dilutions to

determine the unknown virus sample titer using y= mx +b for the trendline equation. The R^2 value should be >0.95 to justify the proper assay setup. Note to include the dilution factor in the final calculation (i.e. if you diluted your purified viral samples 1/100 in step 1 and 1/10/ in Step 3, then the titer of the unknown sample should be multiplied by a factor of 100x10).

Use the Ct values to calculate the viral titer of the viral sample with the following formula:

Titer of unknown sample (GC/ml) = $e^{(Ctx-b)/m}$, Where m is the slope of the line, and b is the y-intercept.

Example: Trendline equation is y= -1.349In(x) + 40.898; Ct if unknown sample = 16.98
Virus titer (GC/ml) = $e^{(16.98-40.898)/-1.349}$ =5.01 x 10^7 GC/ml

Note: Remember to include the dilution factor in the calculation if dilutions of viral particles had been performed.

8. Typical Data of Universal AAV qPCR Performance

The AAV qPCR Performances are listed in Fig. 2, Fig.3 and Table 4. They are only used as reference.

Fig. 2: PCR Amplification Specification

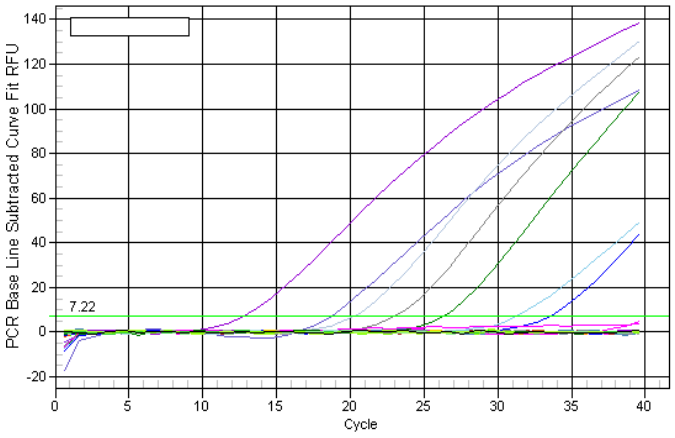

Fig.3. AAV DNA Standard Curve

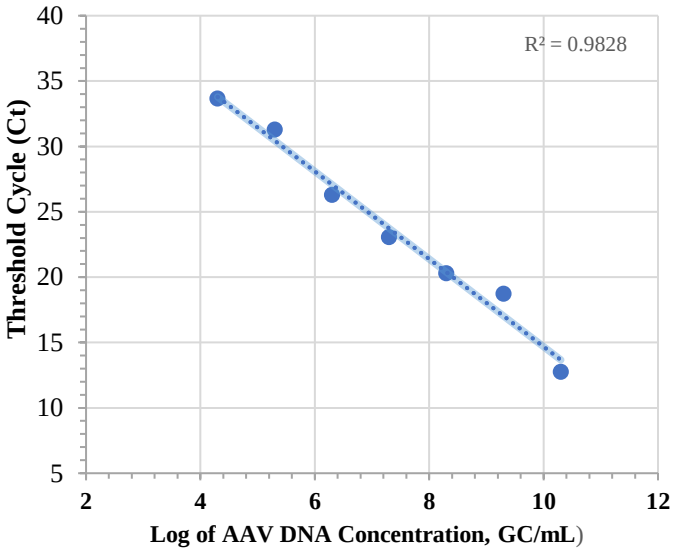

Table 4: AAV DNA qPCR Amplification Sensitivity

AAV DNA (GC/mL)	Threshold Cycle (Ct)
$2x10^{10}$	12.76
$2x10^{9}$	18.72
$2x10^{8}$	20.30
$2x10^{7}$	23.06
$2x10^{6}$	26.30
$2x10^{5}$	31.29
$2x10^{4}$	33.67
Negative	0

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