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Adeno-Associated Virus qPCR Quantification Kit

05/20

(Catalog # K1473-100 Rxns; Store at -20°C)

I. Introduction:

BioVision's Adeno-Associated Virus qPCR Quantification Kit is used for the quantification of adeno-associated Virus. This kit employs a quick DNA extraction step from AAV particles, followed by a qPCR assay. The kit has demonstrated high sensitivity and specificity. Virus samples originating from both virus-producing cell lines or purified viral preparations can be used for determining the titer. Viral DNA is isolated from the sample using the Virus Lysis Buffer provided in the kit. Primers included in the Reagent Mix are then used with the extracted viral DNA, AAV STD1 and STD2 to determine the threshold cycle (C_t) values by qPCR. The C_t values are then used to calculate the viral titer of the adeno-associated virus samples.

II. Application:

- An ideal tool to quantifyAdeno-Associated Virus

III. Key Features:

- Reliable and Ready-to-use
- Results ready in less than 2 h
- **High specificity** and sensitivity
- Minimal non-specific background

IV. Sample Type:

Virus-producing cell lines

V. Kit Contents:

Components	K1473-100 (100 Rxns)	Part Number
Virus Lysis Buffer	1 ml x 2	K1473-100-1
Reagent Mix	1.0 ml	K1473-100-2
STD1 (AAV Standard 1)	300 µl	K1473-100-3
STD2 (AAV Standard 2)	300 µl	K1473-100-4
DNase Reaction Mix	1 ml x 2	K1473-100-5

VI. User Supplied Reagents and Equipment:

- Dye-based 2X qPCR Master Mix

Note: Please select the Master Mix appropriate for your specific instrument. Refer to BioVision's Jade™ Master Mixes Cat. Nos. (M1105-M1108) which come in a range of formulations. Each Master Mix has been optimized for performance according to the QPCR machine and reference dye (Mix (No dye) = without reference dye (ROX) dye, icycler = for icycler, low ROX = for low ROX, ROX = regular ROX). Please click the link https://www.biovision.com/documentation/support/QPCR-Selection-Guide.pdf for selecting the appropriate Master Mix.

- qPCR Thermal Cycler
- PCR tubes
- Nuclease Free Water
- 1X Phosphate Buffered Saline

VII. Shipping and Storage Conditions:

The kit should be stored at -20°C in a non-frost-free freezer upon arrival. Avoid repeated freeze and thaw cycles. All reagents are stable for up to 12 months when stored properly at -20°C.

VIII. Assay Protocol:

1. **Sample Preparation:** For viral samples from a **virus-producing cell line**: collect the culture medium and centrifuge for 5 min at 2000 g to remove cells/debris. Perform DNase I Treatment prior to Viral DNA Isolation step. For **purified virus samples**: dilute the virus to 10^{8-9} GC/ml with 1X Phosphate Buffered Saline and proceed directly to Viral DNA Isolation step (DNase I Treatment not required).
2. **DNase I Treatment**
   a. Set up the following reaction in an Eppendorf tube.

Components	Volume (20 µl reaction)
AAV Sample	2 µl
DNase Reaction Mix	18 µl

   b. Incubate the samples at 37°C for at least 15 min to digest free gDNA, plasmid DNA and unpackaged viral DNA derived from host cells. Incubate at 95°C for 10 min to heat inactivate DNase I.
3. **Viral DNA Isolation**
   a. Combine AAV sample to Virus Lysis Buffer in a 1:1 ratio.
   b. Incubate samples at 70°C for 10 min.
4. **qPCR Set-up**
   Set-up the following reactions on ice in sterile tubes as given below. Prepare triplicates for STD1, STD2, samples with 2X qPCR Mastermix (not provided) in this kit. Mix well and centrifuge briefly. Also set up a Non Template Control Non Template Control (NTC).

Components	Sample	STD1	STD2	NTC
2X qPCR Mastermix	12.5 µl	12.5 µl	12.5 µl	12.5 µl
Viral Lysate (from isolation step)	2.5 µl	-	-	-
STD1	-	2.5 µl	-	-
STD2	-	-	2.5 µl	-
Reagent-mix	10 µl	10 µl	10 µl	10 µl
Nuclease Free Water	-	-	-	2.5 µl
Final volume per reaction	25 µl	25 µl	25 µl	25 µl

5. **qPCR Program**
   Perform qPCR reaction using the following cycling program.

Step	Temperature	Duration	Number of Cycles
Enzyme Activation	95°C	10 min	1
Denaturation	95°C	15 sec	40
Annealing/Extension	60°C	1 min	

IX. Data Analysis

The titer of your sample(s) can be calculated from C_t values by using the following formula:

Titer of viral lysate = $5 \times 10^9/2^{3(C_{tx}-C_{t1})/(C_{t2}-C_{t1})}$

C_{tx} = Average of 3 C_t values of the unknown sample

C_{t1} = Average of 3 C_t values of STD1

C_{t2} = Average of 3 C_t values of STD2

Ensure that dilutions have been accounted for: Dilutions made in Sample Preparation, 10X dilution in DNase I Treatment if it was performed, 2X dilution in Viral DNA Isolation. The final titer will be D (x 10) x 2 x titer calculated, where D stands for the dilution factor performed in Sample Preparation Step 1.

Units: The final titer value obtained is in GC/ml.

X. Related Products:

Product Name	Cat. No.	Sizes
Instant Lentivirus Detection Card	K1470	-10, -20 tests
Lentivirus qPCR Quantification Kit	K1471	100 Rxns
Retrovirus qPCR Quantification Kit	K1472	100 Rxns
Mag-Lentivirus and Retrovirus Purification Kit	K1458	20, 100 Preps
Lentivirus Mini Purification Kit	K1305	-10, -20 preps
Lentivirus Maxi Purification Kit	K1306	-2, -4, -10 preps
PEG Virus Precipitation Kit	K904	-50, -200 preps
Mag-Adenovirus Purification Kit	K1459	-10, -200 preps
Adenovirus Mini Purification Kit	K1300	-10, -20 preps
Adenovirus Maxi Purification Kit	K1301	-2, -4, -10 preps
Retrovirus Mini Purification Kit	K1307	-10, -20 preps
Retrovirus Maxi Purification Kit	K1308	-2, -4, -10 preps
HCV Mini Purification Kit	K1309	-10, -20 preps
HCV Maxi Purification Kit	K1310	-2, -4, -10 preps