

AAV Titer Kit, qPCR

Cat. No. BGT-MPM-109

Store at -20°C.

Product Description

qPCR AAV Titer Kit is a one-step assay which **employs a quick lysis step that is followed by qPCR**. Designed to deliver **high sensitivity and specificity**, the kit ensures minimal non-specific background and better overall performance compared to similar kits on the market.

Product Component	Volume	Part No.
2X qPCR Titer MasterMix	1.25 ml	1
Primer Mix	100 rxn (200 µl)	A
Control DNA	50 µl	B
DNase I Reaction Mix	2 x 1.0 ml	C
Virus Lysis Buffer	800 µl	Buffer-C
ROX Reference Dye	15 µl	P101
Nuclease-Free H ₂ O	2 x 1.0 ml	P100

Protocol

MasterMix contains dye comparable to SYBR Green™ and EvaGreen™. ROX Reference Dye is provided separate from the MasterMix, making this kit universally compatible with most qPCR instruments.

The recommended amount of ROX Reference Dye to be added into the MasterMix may vary depending on the qPCR machine type:

- No ROX equipment: Not needed.
- Low ROX equipment: 1.0 µl/1.25 ml MasterMix.
- High ROX equipment: 11 µl/1.25 ml MasterMix.

1. **Sample Preparation:** For purified high titer viral samples, dilute the virus to 10⁸ GC/ml range with 1X PBS or DMEM. For low viral titer samples, collect viral supernatant for direct qPCR set up.
2. **(Optional) DNase I treatment recommended for crude viral samples:** Add 2 µl of AAV Sample into 18 µl of DNase I Reaction Mix. Incubate samples at 37°C for at least 15 minutes to digest free gDNA, plasmid DNA and unpackaged viral DNA derived from host cells. Incubate at 95°C for 10 minutes to heat inactivate DNase I.

3. **Viral Lysis:** Add 2 µl of the sample preparation (from Step 1 or 2) to 18 µl of Virus Lysis Buffer and incubate at room temperature for 3 minutes. Use the lysed sample for the reaction set up (in Step 5). 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 Dilutions:** Perform five (5) 10-fold serial dilutions of the Standard Control DNA by diluting 5.0 µl DNA into 45 µl Nuclease-free H₂O in each step. Dilutions 1/100 to 1/100,000 will be used for generating the standard curve.
5. **Set-up:** All reactions are recommended to be set-up on ice in duplicates.

Component	Volume
2X qPCR MM	10 µl
Primer Mix	2 µl
Sample, NTC, or Standard DNAs	2 µl
Nuclease-Free H ₂ O	6 µl

6. qPCR cycling conditions:

Step	Temperature	Duration	Cycles
Enzyme Activation	95°C	10 min	1
Denaturation	95°C	15 sec	30
Annealing/Extension	62°C	1 min	

Data Analysis

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 $y = m \ln(x) + b$ from the trendline equation. The R² 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 sample 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 100*10).

$$\text{Virus titer (GC/ml)} = e^{(Ct - b)/m}, \quad \text{where } m \text{ is the slope of the line and } b \text{ is the y-intercept.}$$

Example: trendline equation is $y = -1.349 \ln(x) + 40.898$; Ct of unknown sample = 16.98

$$\text{Virus titer (GC/ml)} = e^{(16.98 - 40.898)/-1.349} = 5.01 \times 10^7 \text{ GC/ml}$$

Dilution	Virus Titer (GC/ml)
1/100	2x10 ⁹
1/1,000	2x10 ⁸
1/10,000	2x10 ⁷
1/100,000	2x10 ⁶