# qPCR AAV Titer Kit

## Cat. No. G931

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. |
|---|---|---|
| BlasTaq™ 2X qPCR Titer MasterMix | 1.25 ml | P889-1 |
| Primer Mix | 100 rxn (200 µl) | G931-A |
| Control DNA | 50 µl | G931-B |
| DNase I Reaction Mix | 2 x 1.0 ml | G931-C |
| Virus Lysis Buffer | 800 µl | LV900-C |
| ROX Reference Dye | 15 µl | P101 |
| Nuclease-Free $H_2O$ | 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.

*See **Rox Machine Compatibility** on our product page under the Documents tab on our website.*

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 $10^8$ 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 $H_2O$ 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 $H_2O$ | 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 $R^2$ 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 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).

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

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

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

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

*Download the **qPCR AAV Titer Calculation Form** from the product page under the Datasheet Tab on our website.*

Applied Biological Materials Inc.● 1-866-757-2414 ● info@abmGood.com ● www.abmGood.com