

qPCR Lentivirus Assay Kit | qPCR assay kit

qPCR Lentivirus Titer Kit

CATALOG #
AAA269072

PRODUCT SYNONYMS

qPCR Lentivirus; qPCR Lentivirus Titer Kit; qPCR assay kit

PRODUCT SPECIFICATIONS

Product Components

Component: BlasTaq 2X qPCR Titer MasterMix
Quantity: 1.25 ml

Component: Primer Mix
Quantity: 100 rxn (200ul)

Component: Standard Control DNA
Quantity: 50ul

Component: Virus Lysis Buffer
Quantity: 800ul

Component: ROX Reference Dye
Quantity: 15ul

Component: Nuclease-Free H₂O
Quantity: 2 x 1.0ml

Protocol

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: 1ul/1.25ml MasterMix.
- High ROX equipment: 11ul/1.25ml MasterMix.

1. Sample Preparation: For purified high titer viral samples, dilute the virus to 107 IU/ml range with 1X PBS or DMEM. For low viral titer samples, collect viral supernatant for direct qPCR setup.

2. Viral Lysis: Add 2ul of the sample preparation (from Step 1) to 18ul of Virus Lysis Buffer and incubate at room temperature for 3 min. Use the lysed sample for the reaction set up (in Step 4). Note: The viral sample has been diluted 1/10, thus take the dilution factor into consideration when calculating the final titer.

3. Standard Control DNA Dilutions: Perform five (5) 10-fold serial dilutions of the Standard Control DNA by diluting 5ul DNA into 45ul Nuclease-free H₂O in each step. Dilutions 1/100 to 1/100,000 will be used for generating the standard curve.

4. Set-Up: All reactions are recommended to be set-up on ice in duplicates.

Component: 2X qPCR MM

Volume: 10ul

Component: Primer Mix

Volume: 2ul

Component: Sample, NTC, or Standard DNAs

Volume: 2ul

Component: Nuclease-free H₂O

Volume: 6ul

5. qPCR Cycling Conditions:

Step: Reverse Transcription

Temperature: 42°C

Duration: 20 min

Cycle(s): 1

Step: Enzyme Activation

Temperature: 95°C

Duration: 10 min

Cycle(s): 1

Step: Denaturation

Temperature: 95°C

Duration: 15 sec

Cycle(s): 30

Step: Annealing/Extension

Temperature: 60°C

Duration: 1 min

Cycle(s): 30

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 with 1/10 dilution from Step 2, then the titer of the unknown sample should be multiplied by a factor of 1000). Virus titer (IU/ml) = $e^{(Ct - b)/m}$, where m is the slope of the line and b is the y-intercept. Example: trendline equation is $y = -1.349 \ln(x) + 40.989$; Ct of unknown sample = 16.98 Virus titer (IU/ml) = $e^{(16.98 - 40.989)/-1.349} = 5.01 \times 10^6$ IU/ml Dilution: 1/100 Virus Titer (IU/ml): 1×10^9 Dilution: 1/1,000 Virus Titer (IU/ml): 1×10^8 Dilution: 1/10,000 Virus Titer (IU/ml): 1×10^7 Dilution: 1/100,000 Virus Titer (IU/ml): 1×10^6
Preparation and Storage	Store at -20°C.

ADDITIONAL INFORMATION

Related Product Information for qPCR assay kit	qPCR Lentivirus Titer Kit is a one-step assay which employs a quick RNA extraction step that is followed by RT-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. ROX reference dye is provided separate from the MasterMix, making this kit universally compatible with most qPCR instruments. MasterMix contains dye comparable to SYBR Green and EvaGreen.
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For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

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Online datasheet: <https://www.aaabiotech.com/assay-kits/qpcr-lentivirus/269072>