



Lentivirus qPCR Quantification Kit

Rev 03/21

(Catalog # K1471-100 Rxns; Store at -20°C)

I. Introduction:

BioVision's Lentivirus qPCR Quantification Kit is a one-step assay by qPCR which does not require additional lysis or reverse transcription (RT) steps, and can be completed under one hour. Our 2X qPCR MasterMix has intrinsic reverse transcriptase activity, thus eliminating the need for an extra reverse transcription incubation step. The kit is designed to deliver high sensitivity and ensures minimal non-specific background. ROX reference dye is provided separately from the MasterMix, making this kit universally compatible with most qPCR instruments.

II. Application:

- An ideal tool to quantify Lentivirus

III. Key Features:

- Reliable and Ready-to-use
- Results ready in 1 h
- **High specificity** and sensitivity
- Minimal non-specific background

IV. Sample Types:

Virus-producing cell lines or purified viral preparations

V. Kit Contents:

Components	K1471-100 (100 Rxns)	Part Number
2X qPCR MasterMix	1.25 ml	K1471-100-1
Primer Mix	200 µl	K1471-100-2
Standard Control DNA	50 µl	K1471-100-3
ROX Reference Dye	15 µl	K1471-100-4
Nuclease-Free Water	2 x 1.0 ml	K1471-100-5

VI. User Supplied Reagents and Equipment:

- qPCR Thermal Cycler
- PCR tubes
- 1X Phosphate Buffered Saline or DMEM

VII. Shipping and Storage Conditions:

The kit should be stored at -20°C 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:

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 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 samples to 10⁷ IU/ml range with 1X Phosphate Buffered Saline or DMEM. For low viral titer samples, collect viral supernatant for direct qPCR.
2. **Standard Control DNA Dilutions:** Perform five (5) 10-fold serial dilutions of the Standard Control DNA by diluting 5 µl DNA into 45 µl Nuclease-free Water in each step. Dilutions 1/100 to 1/100,000 will be used for generating the standard curve.
3. **Set-up:** All reactions are recommended to be set-up on ice in duplicates.

Components	Volume
2X qPCR Mastermix	10 µl
Primer Mix	2 µl
Sample, NTC, or Standard DNA	2 µl
Nuclease-Free Water	6 µl

4. qPCR cycling conditions:

Step	Temperature	Duration	Number of Cycles
Enzyme Activation	95°C	10 min	1
Denaturation	95°C	15 sec	35
Annealing/Extension	60°C	1 min	

IX. 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 titers of the standards are:

Dilution	Virus Titer (IU/ml)
1/100	5 x 10 ⁸
1/1,000	5 x 10 ⁷
1/10,000	5 x 10 ⁶
1/100,000	5 x 10 ⁵

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, then the titer of the unknown sample should be multiplied by a factor of 100).

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

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

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

X. Selection Guide For ROX and qPCR Machine Type:

MACHINE TYPE	qPCR MACHINE
High ROX Machines	<ul style="list-style-type: none"> • ABI® 7000, 7300, 7700, 7900, 7900HT, StepOnePlus™, StepOne™, OpenArray, PRISM™ Sequencing Detection Series • Biometra TOptical • Fluidigm BioMark™ • Wafergene SmartChip System • TianLong TL998 System
Low ROX Machines	<ul style="list-style-type: none"> • ABI® 7500, 7500 Fast, Vii™ 7, QuantStudio, QuantStudio 3/5/6/7 • BioGene InSyte™ • Illumina Eco • Analytikjena qTower Series • Stratagene® Mx3000, Mx3005, Mx4000
No ROX machines	<ul style="list-style-type: none"> • BioRad® CFX96, CFX384, Chromo4™, CFX Connect™, Opticon 2, MiniOpticon™ • Roche LightCycler® (480, 1536, Nano) • MJ Research Opticon™, Opticon™ 2, Chromo® 4 • Eppendorf™ Realplex 4 • BioGene SynChron™ • Corbett Rotor-gene® (3000, 6200, 62H0, 6500, 65H0, 6600) • Eppendorf Mastercycler® realplex (s, 4, 4s), Pro (S, 384), Nexus (gradient, eco, flat) • Cepheid SmartCyler, GeneXpert • Enigma™ ML • Idaho LightScanner® (24, 32), RapidCycler®2, R.A.P.I.D (LT, LT Food), RAZOR EX, JBAIDS • Qiagen Rotor-Gene™ (Q, 6000) • Takara Dice™ • Thermo Scientific PikoReal • DNA-Technology DT96, DTlite, DT-322 • Bioer LineGene (3310/3320, K FQD-48A, I, II, 9620, 9640, 9660, 9680) • Bioneer Exicycler™

XI. Related Products:

Product Name	Cat. No.	Sizes
Instant Lentivirus Detection Card	K1470	-10, -20 tests
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
Retrovirus Mini Purification Kit	K1307	-10, -20 preps
HCV Mini Purification Kit	K1309	-10, -20 preps

FOR RESEARCH USE ONLY! Not to be used on humans.