BlueKit™
Powered by Hillgene

Ver202502

Instructions for Use
Lentiviral Vector RNA Copy Number Detection kit

(Cat. No. HG-VR001)

CONTENTS

1. Product Instruction

During the process development of lentiviral vector preparation, attention needs to be paid to the establishment of quality standards for viral vectors, including viral titer, residual host cell DNA, residual host cell protein, mycoplasma, and etc. Among them, viral titer is essential in terms of virus use as well as viral infection of cells, and is a critical quality attribute (CQA) for lentiviral vector manufacturing. Titer assays include physical titer (the amount of viral particles) and transduction titer (the amount of infectious particles of the viral vector). Quantitation of vector-specific proteins/nucleic acids can be used for estimation of the number of vector particles (Physical Titer), that is, the total particle number and genomic titer represent the physical number of viruses as physical titer. For example, physical titer of HIV-1 derived lentiviral vectors can be calculated based on the p24 protein in the viral vector sample by ELISA, or genomic RNA copy number of the vector by qPCR.

The kit uses TaqMan fluorescent probe for qPCR to get physical titer by detecting the copy number of the vector genome RNA, with rapid detection, high specificity, reliable performance for universal lentiviral vectors.

The kit is equipped with quantitative reference materials constructed by Hillgene.

Detection range：2×10^{2}copies/μL ~ 2×10^{7}copies/μL

2. Scope

The kit provides a rapid and specific detection of physical titers in lentiviruses.

3. Kit Specification

Product name	Cat. No.	Product Specification
Lentiviral Vector RNA Copy Number Detection kit	HG-VR001	100 Reactions/Kit

4. Kit Components

Components	Specification	Storage condition
Vector RNA Quantitative Standard	50μL×1	-20°C
(2×10^{8} copies/μL)	50 μL×1 vial	
Vector Gene Primer & Probe MIX	550 μL×1 vial	
2x qPCR Reaction Buffer	1.2 mL×1 vial	
DNA Diluent	1.5 mL×3 vial	
ROX High	50 μL×1 vial	
ROX Low	50 μL×1 vial	

Note: real-time quantitative fluorescence PCR instruments adapted to the kit includes but not limited to ABI7500, BioRad CFX96, Bioer FQD-96A, Roche Light Cycler 480 and so on.

5. Storage Condition and Shelf Life

Storage conditions are shown in the table above and shelf life is 18 months. Unused kits after opening are still kept under the required storage conditions.

6. Definition or Terminology

- No Template Control (NTC) refers to a sample in a PCR reaction that intentionally lacks any positive or negative template, generally RNase-free water or diluent.
- Negative Control Sample (NCS) refers to a known uninfected sample that that is not expected to produce a positive result after extraction and amplification, and emphasizes a clear expected result with the consistent treatments and samples compared with the test group.
- Extraction Recovery Control (ERC) refers to the addition of a standard (component to be measured) of known content to a blank sample or some background of known content, and use an established method to detect the ratio of the content (measured value) to the added value.

7. Reagents, Consumables and Equipment to Be Prepared

Please prepare the following reagents, consumables and equipment before the experiment:

- RNase-free water
- Clean bench/Biosafety cabinet
- 1.5 mL or 2 mL sterile low adsorption centrifuge tubes
- 96-well qPCR plates or 8-tube strip adapted to PCR instruments
- 1000 µL, 200 µL, 10 µL, etc. sterile low adsorption filtered tips
- PCR instrument
- Centrifuge
- 1000 µL, 200 µL, 10 µL, etc. pipettes
- Water bath/Dry bath
- Viral RNA Extraction Kit
- Reverse Transcription Kit
- Vortex mixer

8. Precautions

- This kit has been validated for stability (freeze-thaw and other factors) and does not require aliquoting.
- Negative and positive samples (reference and samples to be tested, etc.) needs to be prepared in different areas, and the preparation personnel need to be fully dressed, wearing masks, gloves and clean clothes.
- Change tips for every spiking step to avoid cross contamination, and avoid prolonged opening of the cap.
- The kit must be within the validity period when being used.
- All components of the kit are recommended to be used after melting in a cold environment.

- Only by strictly following the instructions and using all the reagents that come with the kit can you ensure optimal testing results.
- Sample pre-treatment is recommended to complete on the same day as far as possible, and the subsequent qPCR assay is recommended to perform immediately after purification to ensure the accuracy of the results.
- The final test results are closely related to the validity of the reagents, the operator’ s practices and the test environment.
- The company is only responsible for the kit itself, not for the consumption of samples caused by the use of the kit. Please take into account the possible sample usage and reserve sufficient samples before use.
- This kit is intended for in vitro research use only and not for clinical diagnosis.

9. Operating Procedures

9.1. Detection flow chart

TotalTime:4 h

9.2. Preparation

9.2.1. Proteinase K shall be stored at -20° C if it is a long-term storage.

9.2.2. Check Buffer RLC for crystals or sediment before use, and if any, re-dissolve Buffer RLC in a water bath at 56° C.

9.2.3. Dilute the lentivirus 10-fold for use in the assay; take 50 µL of RNase-free water as NCS for sample pre-treatment; take 50 µL of RNase-free water as NTC for PCR assay.

9.3. Sample DNA digestion

Components	Volume	Final concentration
Lentivirus sample	50 µL	NA
10 × Reaction Buffer	20 µL	1×
DNase Ⅰ (RNase Free)	10 µL	1 U
RNase Free Water	120 µL	Top up to 200 µL

9.3.1. Pipette samples 10 times, centrifuge 5 seconds and digest at 37° C for 20 minutes.

9.3.2. At the end of digestion, centrifuge sample for 10 seconds, add 10 µL of EDTA to a final concentration of 10 mM, and inactivate DNase I at 65° C for 10 minutes.

9.4. RNA extraction

9.4.1. Add 500 µL of Buffer RLC, 20 µL of Proteinase K to the digested sample, vortex and shake for 5 seconds, then place at room temperature and shake for 10 minutes in a thermostatic vortex mixer.

9.4.2. Centrifuge the centrifuge tube and add the solution obtained in the previous step to the Spin Columns that have

been loaded into RNase-Free Centrifuge Tube. Centrifuge at 13,400 g for 1 minute, discard the waste from the RNase-Free Centrifuge Tube and place the column back into it.

9.4.3. Add 500 µL of Buffer PGWT to the spin column, centrifuge at 13,400 g for 1 minute, and discard the waste from the RNase-Free Centrifuge Tube and place the column back into it.

9.4.4. Add 500 µL Buffer GWT2 to the spin column, centrifuge at 13,400 g for 1 minute, discard the waste from the RNase-Free Centrifuge Tube and place the column back into it.

9.4.5. Centrifuge at 13400 g for 2 min and discard the waste in RNase-Free Centrifuge Tube. Place the spin column at room temperature for 2 minutes for drying.

9.4.6. Place the spin column in a new RNase-Free Centrifuge Tube, add 50 µL of RNase-Free Water to the center of the spin column membrane without touching the membrane, leave at room temperature for 2 minutes, then centrifuge at 13,400g for 1 minute and collect the nucleic acid solution. Place it at -80° C for long-term storage.

9.5. Reverse transcription

9.5.1. After placing the reagents on the ice box to melt, prepare the reaction buffer on the ice box according to the table below:

Components	Usage volume
Total RNA	5 µL
5×StarLighter Script RT Super Mix	4 µL
gDNA Removal	1 µL
H_2O (Nuclease-Free)	10 µL

9.5.2. All operations are kept on ice as much as possible, and after gentle pipetting, centrifuge for 5 seconds for gDNA removal and reverse transcription, and the reaction system is shown in the table below.

Reaction system

Temp.	Reaction time	Purpose
37°C	5 min	Genome removal
50°C	15 min	gDNA Removal inactivation & reverse transcription
85°C	1 min	Reaction termination

9.5.3. After the reaction, store at 4°C or -20°C (long term), and cDNA should avoid freeze/thaw. cDNA is added as a template either directly or after dilution for Real Time PCR. Note: Do not add more than 20% of the reverse transcription reaction buffer to the Real Time PCR reaction buffer. Excessive addition will result in inefficient Real Time PCR reaction and inaccurate quantification.

9.5.4. Take 20 µL of cDNA sample, add 30 µL of RNase-free water, ready for use after pipetting.

9.6. CR amplification

9.6.1. Preparation of standard solution

Melt the standard and DNA diluent in the refrigerator at 4°C; after melting completely, mix them upside down 20 times, then centrifuge them at 6000rpm for 5 seconds in a compound rotor centrifuge, and perform a serial dilution using the DNA diluent to dilute the RNA quantitative reference materials (2E+08 copies/μL) to several gradients of 2E+07 copies/μL, 2E+06 copies/μL, 2E+05 copies/μL, 2E+04 copies/μL, 2E+03 copies/μL, and 2E+02 copies/μL. The specific operation is as follows: take six of 1.5 mL centrifuge tubes labelled STD1, STD2, STD3, STD4, STD5, STD6, and add 90 μL of DNA dilution to each of them. Refer to the following table for the dilution operation (the way to dilute the standards is pipetting, 20 times continuously at a uniform speed):

Diluent tubes	Dilution volume	Concentration (copies/μL)
ST1	10μL reference materials + 90 μL DNA diluent	2E+07
ST2	10 μL ST1 + 90 μL DNA diluent	2E+06
ST3	10 μL ST2 + 90 μL DNA diluent	2E+05
ST4	10 μL ST3 + 90 μL DNA diluent	2E+04
ST5	10 μL ST4 + 90 μL DNA diluent	2E+03
ST6	10 μL ST5 + 90 μL DNA diluent	2E+02

9.6.2. Preparation of ERC Solution

Take 20 μL of the purified solution of the sample to be tested, add 20 μL of ST2, and mix well as the ERC solution. (The preparation of the ERC solution can be adjusted according to the situation)

9.6.3. Preparation of qPCR reaction buffer

9.6.3.1. Calculate the amount of qPCR MIX required according to the number of reaction wells, and prepare qPCR MIX according to the number of standard samples to be tested and the number of samples to be tested (usually 3 duplicate wells). Calculate the total amount of qPCR MIX according to the number of reaction wells: qPCR Mix = (number of reaction wells + 2 or 3) × 15μL ("2 or 3" considers the loss during preparation)

9.6.3.2. Prepare the qPCR Mix according to the table below.

Components	Reaction volume in a single well (μL)
PCR Reaction Buffer	10
Primer/Probe MIX	4.6
ROX	0.4
Total volume	15

Note: For instruments that do not require ROX, RNase-free water can be the substitution. and please refer to the following table to find the ROX adapted to the different instruments; if failed, please reach out to us or instrument manufacturers.9.6.4.Example Layout of Reaction Wells:

Instruments	ROX reference dye
ABI 5700, 7000, 7300, 7700, 7900HT Fast, StepOne, StepOne Plus	ROX High
ABI 7500, 7500 Fast, ViiA7, QuantStudio 3 and 5, QuantStudio 6, 7, 12k Flex. Stratagene MX3000P, MX3005P, MX4000P	ROX Low
Bio-Rad CFX96, CFX384, iCycler iQ, iQ 5, MyiQ, MiniOpticon, Opticon, Opticon 2, Chromo4. Roche Applied Science LightCycler 480, LightCycler 2.0; Lightcycler 96. Eppendorf Mastercycler ep realplex, realplex 2 s. Qiagen Corbett Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000. Thermo Scientific PikoReal Cycler. Cepheid SmartCycler. Illumina Eco qPCR	No ROX

9.6.3.3.Each reaction well is spiked as shown in the table:

Standard curve	15 μL qPCR Mix+5 μL ST1/2/3/4/5/6
Control	15 μL qPCR Mix+5 μL NTC/NCS/ERC
Sample to be tested	15 μL qPCR Mix+5 μL 待测样本 15 μL qPCR Mix+5 μL sample to be tested

9.6.3.4.3.replicates of each sample are made, and centrifuge them at 6000 rpm for 10 seconds in a compound rotor centrifuge to remove air bubbles from the 8-tube strip. The plate layout is as follows:

/	1	2	3	4	5	6	7	8	9	10	11	12
A	ST1	ST1	ST1	S1	S1	S1						NTC
B	ST2	ST2	ST2	S2	S2	S2						NTC
C	ST3	ST3	ST3	S3	S3	S3						NTC
D	ST4	ST4	ST4									
E	ST5	ST5	ST5	ERC-S1	ERC-S1	ERC-S1						
F	ST6	ST6	ST6	ERC-S2	ERC-S2	ERC-S2						NCS
G				ERC-S3	ERC-S3	ERC-S3						NCS
H												NCS

Note: ST stands for Standard, NTC stands for No Template Control, NCS stands for Negative Control Sample, ERC stands for Extraction Recovery Control, and S stands for sample to be tested.

9.6.4.Standard Cycling Parameters:

Step	Temp.	Time	Cycle	Note
Initial denaturation	95°C	5 min	1	Initial denaturation
Denaturation	95°C	15 sec	40	NA
Annealing/extension	58°C	1 min		Fluorescence signal acquisition
	72°C	12 sec		NA

9.6.5.Create an experimental reaction plate, click Select Fluorophores to select Fluorescent FAM; in the Reaction Plate Chart, select the sample well, and under the category of Sample Type, drop down to select Unknow, select FAM as the fluorescent reporter, enter Vector in Target Name, and enter repetition times and Sample Name of each sample. (Note: If the instrument requires to set the fluorescence quencher and reference fluorescence, select fluorescence quencher as None, and reference fluorescence as ROX).

9.6.6.In the Reaction Plate Chart, select the standard well, under the category of Sample Type, drop down to select Standard, select FAM as the fluorescent reporter, and name the Target Name as ZL-DNA; enter repetition times and Sample Name of each dilution gradient. Assign the value of 2E+07 copies/μL, 2E+06 copies/μL, 2E+05 copies/μL, 2E+04 copies/μL, 2E+03 copies/μL and 2E+02 copies/μL to the Concentration column of STD1, STD2, STD3, STD4, STD5, and ST6.

9.6.7.Click "Start Run" to run the PCR assay.

10. Data Analysis

Take BIO-RAD CFX96 qPCR instrument as an example.

10.1.Data Analysis：

10.1.1.At the end of the reaction, the instrument automatically sets the baseline and threshold. Thresholds can be adjusted based on experience to improve linearity and amplification efficiency and to optimize variations in 3 replicate wells.

10.1.2.Click Quantitation on the data analysis window to read the Slope, Intercept, Effect and R2 of the standard curve.

10.1.3.In the Quantitation Data window, the assay value of NTC, and the sample to be tested can be read in the SQ Mean column, with the unit of copies/μL.

10.1.4.Click "Tools > Report" or click the Report on the toolbar in the Data Analysis window to bring up the Report window. In the Options list, tick Head, Run Settings, Quantitative Data, and tick Notes to enter notes. In the options pane, enter the title, adjust the font format, or call the previously saved template. Click "Update Report" to update the preview, and if there is no error, click "File > Save" to save the results as PDF format.

10.1.5.Equation：

10.1.5.1. Vector copy number (copies/mL) = assay result (copies/μL) * cDNA dilution factor * virus dilution factor * 1000

10.1.5.2. lentivirus physical titer (VG/mL) = vector copy number/2

Note: there are two strands of positive-sense RNA in one lentiviral particle.

$$\text{Recovery\%} = \frac{(\text{detection concentration of spiked sample} \times \text{total volume}) - (\text{detection concentration of test sample} \times \text{test sample volume})}{\text{theroetical concentration of spiked sample} \times \text{volume of spiked sample}} \times 100\%$$

10.2.System Suitability

10.2.1.The coefficient of variation (CV) of Ct values between three replicate wells shall be ⩽ 5%, except for the wells with Ct values > 35.

10.2.2.R2 ⩾ 0.990, PCR amplification efficiency shall be "Effective: 85% ~ 110%"

10.2.3.NTC shall have no Ct value or have a Ct value which is 2 Ct values greater than lower limit of quantification (LLOQ) in the standard curve.

10.2.4.The Ct value of NCS shall be greater than the Ct value of lower limit of quantification (LLOQ) in the standard curve;

10.2.5.The recovery of ERC shall be within 50%~150%.

11. Example Diagram (using the Bio-Rad as an example)

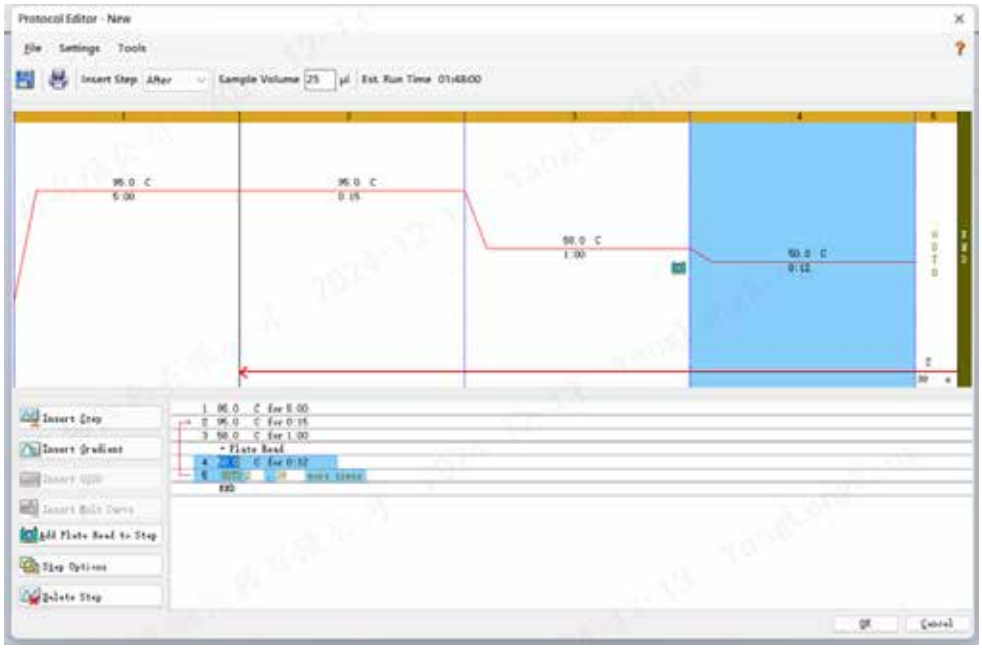

Figure 1: Example diagram of PCR amplification protocol

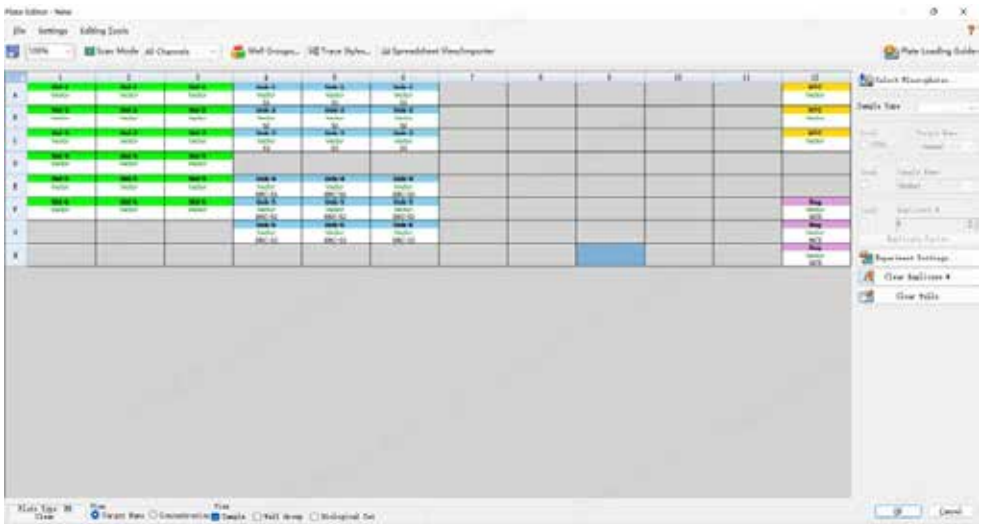

Figure 2: Example diagram of PCR amplification plate layout

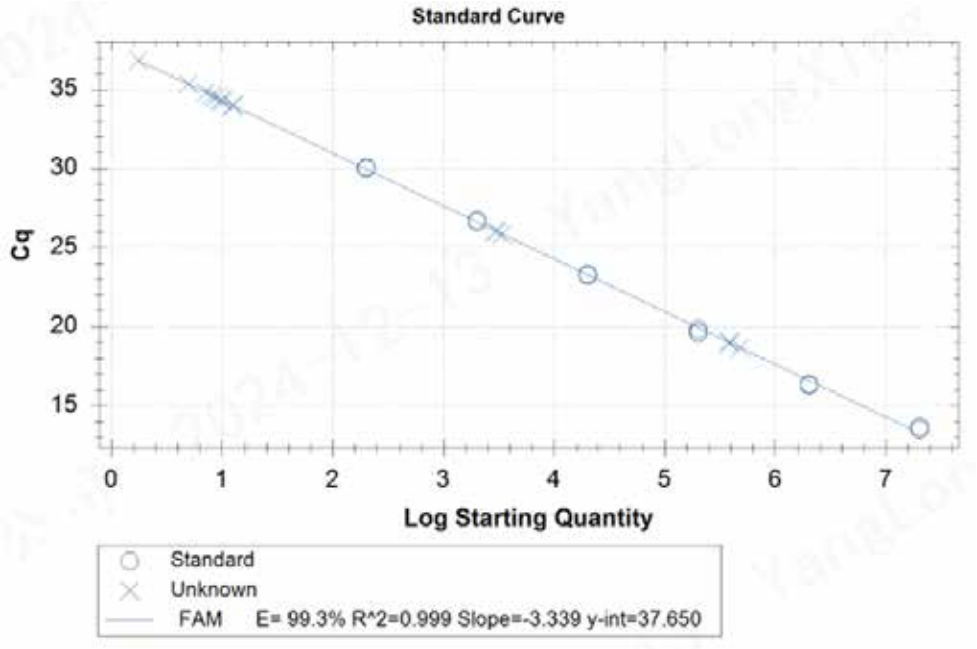

Figure 3: Example diagram of standard curve

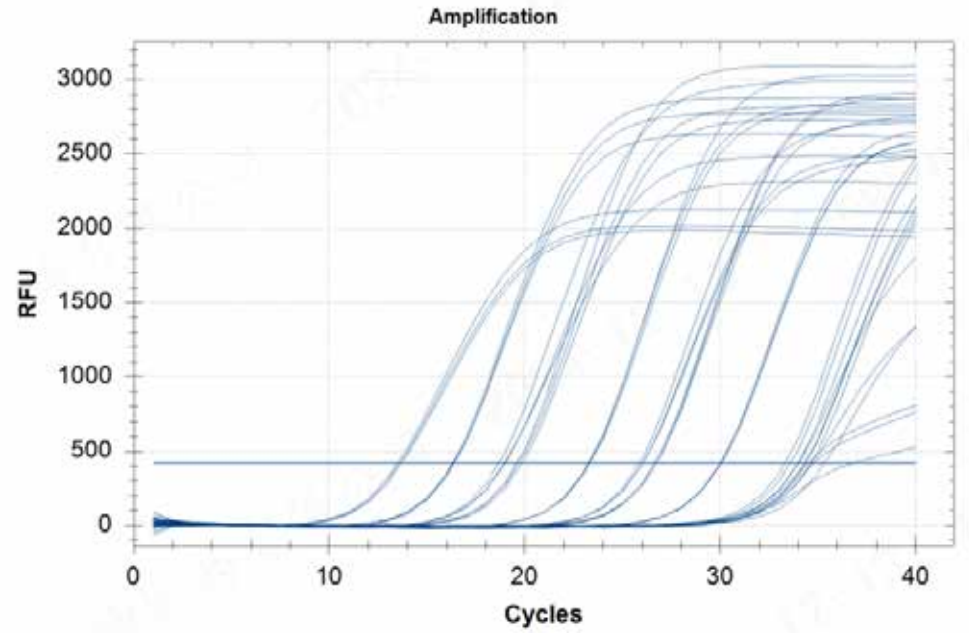

Figure 4: Example diagram of amplification curve

12. Troubleshooting

No.	Troubles	Possible Reasons	Solutions
1	No Ct value	Incorrect PCR protocol settings, and wrong procedure for detecting fluorescent signals.	Check if fluorescence selection and read position in the protocol settings is correct
		Primer or probe degradation	Use PAGE electrophoresis to detect if primer and probe are degraded.
		Possible degradation of the template or insufficient loading volume	The introduction of impurities in sample preparation and multiple freeze-thaw cycles should be considered if template is degraded
2	Values are out of the standard range	Calculation error when preparing the reaction buffer	Review the calculations several times for system preparation
3	Poor standard curve	DNA quantitative reference material dilution, mixing or spiking errors, so that the reference materials fail to show a gradient.	Pipetting equipment should be accurate, the liquid does not hang on the wall when pipetting, the liquid should be well mixed before each step of transfer (check the ROX line for abnormality), the dilution factor should be reasonable, and attention should be paid to whether the liquid is transferred according to the specified volume when pipetting.
		Degraded reference materials	Freeze-thaw cycles of reference materials should be within the specified number of times.
		Presence of inhibitors in the template	Check ROX line for abnormalities and re-dilute template.
4	Late Ct value	Degradation of various PCR reaction components or insufficient spiking volume.	Check ROX lines for abnormalities, run gels to verify degradation of reaction components, repeat experiments at reduced dilution factors
		Presence of inhibitors in the template	Check ROX line for abnormalities and re-dilute template.
5	NCS amplification with signals	Contamination of reaction system components (e.g., DNA diluent)	During the experiment, repeat the experiment with a new Mix; prepare the reaction system on a clean bench.
		Cross-contamination between samples or product contamination; aerosol contamination due to operating environment	Strict zoning of laboratories to reduce aerosol contamination; use of filtered tips; treatment of the operating environment or replacement with a new operating environment, pipettes, tips, etc.
		Fluorescent dye residue on instrument or PCR tube walls	Instrument cleaning, with background testing and calibration; replace tubing, and avoid fluorescent dye contamination when using
6	Abnormal amplification curves	High concentration of the template or degraded template or inadequate mixing and dissolving of the system; degraded fluorescent dye;	Check ROX lines and corresponding multicomponent lines for anomalies, and re-dilute

No.	Troubles	Possible Reasons	Solutions
6	Abnormal amplification curves	Problems such as consumable airtightness cause the liquid to evaporate without collecting well at the bottom of the tube; operational problems such as bubbles caused by spiking with pipettes.	Check tubing’ s airtightness before starting the machine, check carefully whether there are air bubbles left in the reaction tube, and check whether the volume of liquid is normal when it is taken out at the end of the protocol.
		Curve anomalies caused by improper instrument settings	Improper baseline settings, e.g. broken or downhill amplification curve: the baseline endpoint is larger than the Ct value. Decrease the baseline endpoint (Ct value - 4) and re-analyze the data.
7	Low amplification efficiency	If fluorescent dye is degraded	Check ROX lines and corresponding multicomponent lines for anomalies, and re-dilute
		Presence of PCR reaction inhibitors in the system	Inhibitors are introduced when the template is added, so the template should be diluted moderately before adding to the reaction system to reduce the effect of inhibitors.
		DNA quantitative reference material dilution, mixing or spiking errors	Pipetting equipment should be accurate, the liquid does not hang on the wall when pipetting, the liquid should be well mixed before each step of transfer (check the ROX line for abnormality), the dilution factor should be reasonable, and attention should be paid to whether the liquid is transferred according to the specified volume when pipetting.
8	High amplification efficiency	Non-specific amplification occurs and the concentration of template in the reaction system is too high	Delete the reaction well with the highest concentration and re-analyze the standard curve; re-dilute the template
9	Unsmooth amplification curve	Inadequate mixing of reagent	Liquids should be well mixed before each transfer step (check the ROX line for abnormality)
		Presence of inhibitors in the template	Re-dilution of the template
		The fluorescent signals interfere with each other	Check if the instrument model matches with each other and if the fluorescent signal of the kit is compatible with the instrument model.
10	Large gap in CT values	Inconsistent threshold settings; inconsistent instrument	Unified threshold and instrument comparison data; the targets and components of nucleic acid detection products vary in the market, so there will be differences between CT values of the standard curve of different kits, and national standards can be used as an inhouse control for accuracy of values.

13. Contact Information

Address: No. 1463 Wuzhong Avenue, Yuexi Street, Suzhou, Jiangsu Province, China

Postal Code: 215104

Phone: 400-900-1882

E-Mail：info@hillgene.com

After-Sales Email: technical@hillgene.com

Website：https://www.hillgene.com

14. Buyer Notice

Our products are for research use only. They shall not be used for any other purpose, including, but not limited to, human, therapeutic or diagnostic use or any commercial use. Our products shall not be transferred to third parties, resold, modified for resale, or used to manufacture commercial

Attachment 1: Safety Precautions

◆ General Instructions

Failure of the user to use this product in the manner described in this manual may result in personal injury or damage to instruments or equipment. Ensure that personnel using this product have received the general laboratory safety operation instructions and the safety information provided in this document.

(1) Before using the instrument or equipment, read and understand the safety information in the user documentation provided by the instrument or equipment manufacturer.

(2) Before handling chemicals, read and understand all applicable safety data sheets (SDSs) and use appropriate personal protective equipment (gloves, protective clothing, goggles, etc.).

◆ Biological hazard

(1) Biological samples, such as tissues, body fluids, infectious agents and blood of humans and other animals, have the potential to transmit infectious diseases. Therefore, perform all work in a safety equipment such as biosafety cabinets. Safety equipment also includes personal protective equipment such as gloves, coats, work clothes, shoe covers, boots, respirators, face masks, safety glasses, or goggles.

(2) Individuals should be trained according to local regulations and company/institutional requirements before using materials that may be biohazardous.

◆ Hazardous waste (from instruments)

(1) Waste from the instrument is potentially hazardous. Follow the guidelines in the preceding "Biological hazard" warning.

Attachment 2: More Products (refer to https://www.hillgene.com)

Type	Product Name	Cat. No.
Lentivirus testing		HG-HD001
	Human Residual DNA Detection Kit (Probe-based qPCR)	HG-HD001
	Human Residual DNA Segment Analysis Kit (Probe-based qPCR)	HG-HF001
	E. coli Residual DNA Detection Kit (Probe-based qPCR)	HG-ED001
	Plasmid Residual DNA Detection Kit (Probe-based qPCR)	HG-ZL001
	Residual Host SV40LTA & E1A Detection Kit (Multiple Probe-based qPCR)	HG-EA001
	Nuclease ELISA Detection Kit	HG-BE001
	BSA ELISA Detection Kit	HG-BS001
	Trypsin ELISA Detection Kit	HG-TR001
	PG13 Residual DNA Detection Kit (Probe-based qPCR)	HG-PG001
	Residual Host Cell DNA (Microbead Method) Sample Pre-treatment Kit	HG-CL100
	Blood/Tissue/Cell Genomic DNA Extraction Kit	HG-NA100
	Lentivirus titre p24 ELISA kit	HG-P001L
	293T HCP ELISA Detection Kit	HG-HCP001
Lentivirus packaging	Serum-free Suspension Lentivirus Rapid Preparation Kit	HG-HIV-CUL-001
	CD-19 CAR-T Lentivirus	HG-CT1901
	CD-19 CAR-NK Lentivirus	HG-CN1901

CELL THERAPY
INNOVATION INSPIRED

BlueKit™
Powered by Hillgene

Welcome to order

Jiangsu Hillgene Biopharma Co.,Ltd

A : Avenue de l'Hôpital 11, 4000 Liège (Sart-Tilman)
T : 44 7718426057
E : info@hillgene.com
W : www.bluekitbio.com

bluekitbio.com

en.hillgene.com