BlueKit™
Powered by Hillgene

Ver202501

Instructions for Use of Lentivirus Titer p24 Rapid ELISA Detection Kit

(Cat.No. HG-P001L)

CONTENTS

1. Product Instruction

In the process of developing lentiviral vector manufacturing techniques, it is crucial to establish quality standards for the viral vector. These include testing indicators such as lentivirus transfection titer, residual host cell DNA, residual host cell proteins, and mycoplasma. Measuring lentivius titer is vital for using the virus and infecting cells, making it a critical quality attribute (CQA) of lentiviral vector production. Viral titer testing includes: Physical titer (total number of viral vector phsi particles); Transduction titer (number of infectious viral vector particles). Physical Titer: Quantification of vector-specific proteins or nucleic acids can estimate the number of vector particles, representing the physical titer. For instance, lentiviral vectors derived from HIV-1 can measure physical titer via: ELISA is a test for detecting the p24 protein in viral vector samples; qPCR is a testing for measuring the RNA copy number of the vector genome.

This kit utilizes a double antibody sandwich enzyme-linked ELISA method to detect the total p24 content in test samples, converting it to physical titer. The microplate is pre-adding with anti-HIV-1 p24 capture antibodies. Standards and test samples are added to the wells, followed by anti-HIV-1 p24 secondary antibodies. The mixture is incubated at room temperature to form an antibody-antigen-secondary antibody complex. After washing off unbound substances, HRP catalyzes the substrate TMB, producing a blue color. Absorbance is measured at 450 nm ~ 630 nm using a microplate reader. A standard curve is used to calculate p24 content in test samples.

Detection Range:1.37 ng/mL ~ 1000 ng/mL

2. Function

This kit is suitable for the rapid determination of p24 protein content in HIV-1-based lentiviral vectors.

3. Kit Specifications

Product Name	Cat No.	Product Specification
Lentivirus Titer p24 Rapid ELISA Detection Kit	HG-P001L	96T

4. Kit Contents

Contents	Specification	Storage Condition
Coated Plate	1 × 96 wells	2 ~ 8°C
Anti HIV-1 p24+HRP	6 mL	
HIV-1 p24 Standard	S1~S7,S0	
Virus Lysis	6 mL	
Sample Diluent Buffer	50 mL	
10×PBST Wash Buffer	50 mL	
Color Reagent	12 mL	
Stop Solution	12 mL	

Contents	Specification	Storage Condition
Plate Sealer	5	
Instructions for Use (IFU)	1	

5. Storage Conditions & Expiration Date

Refer to the table in the kit instructions. The kit has a 12-month shelf life. Opened kits should be stored under the recommended conditions to maintain efficacy.

6. Abbreviation and Definition

- ELISA (Enzyme-Linked Immunosorbent Assay): A technique utilizing the specific binding of antigens and antibodies. Immobilized antibodies or antigens retain immunological activity. When test samples are added, enzyme-labeled antibodies form immune complexes with immobilized antibodies and sample antigens. The enzyme substrate generates a colorimetric signal for quantitative measurement of the target molecule in the sample.
- p24: A marker protein abundant in lentiviral capsids.
- Physical Titer: Titer calculated based on total p24 protein content. Conversion formula:
- 1 LP contains 8×10^{-5} pg of p24.
- 1 ng of p24 $\approx 1.25 \times 10^{7}$ LPs.
- Typically, 100~1000 LPs $\approx$ 1 IFU.
- 10^{7} IFU/mL $\approx 10^{9} \sim 10^{10}$ LP/mL or 80 ~ 800 ng p24/mL
- CV (Coefficient of Variation): The ratio of standard deviation to the mean.
- ERC: Extraction Recovery Control
- NC: Negative control.

7. Additional Requirements

Before starting the experiment, prepare the following:

- Biosafety cabinet
- Constant temperature shaker
- Microplate reader (equipped with 450 nm and 630 nm wavelengths)
- pipette tips (e.g., 1000 µL, 200 µL, 10 µL)
- Pipettes (1000 µL, 200 µL, 10 µL, etc.)
- Centrifuge
- Vortex mixer
- Deionized water
- Absorbent paper

8. Operation Procedure

8.1. Operation Procedure Figure.

The Whole Processes Take About 1 Hours.

8.2. Preparation

8.2.1. Turn on the UV lamp in the biosafety cabinet for 30 minutes.

8.2.2. Remove the kit from the 2 ~ 8° C refrigerator and allow it to equilibrate to room temperature for at least 30 minutes. Ensure all necessary reagents for the current experiment are available. Mark the opening date on the kit upon first use. Prioritize previously opened kits of the same batch; Do not mix reagents from different batches. Prepare the required Coated Plate for the experiment. Remove unused sections from the microplate, seal them, and store at 2 ~ 8° C as per the instructions for future use.

8.3. Preparation for Wash Buffer: Dilute the 10× PBST Wash Buffer with deionized water to prepare a 1× Wash Buffer. Mix well.

8.4. Preparation for Test Sample: Dilution of test samples should be performed in the biosafety cabinet using the Sample Diluent Buffer. Before dilution or loading, ensure the test samples are completely dissolved and equilibrated to room temperature. Mix thoroughly by pipetting the sample at 90% of the sample's volume for 20 times at a constant speed or vortex at a low speed. Adjust the dilution factor based on actual concentrations.

Note: The dilution factor for a single step should not exceed 10-fold.

8.5. Preparation for Standard Solution:

HIV-1 p24 Standard	Standard Concentration(ng/mL)
S1	1000
S2	333.33
S3	111.11
S4	37.04
S5	12.35
S6	4.12
S7	1.37
S0	0

8.6. Prepare an internal control solution (200 ng/mL) by adding 20 µL of 1000 ng/mL standard (S1) to 80 µL of Sample Diluent Buffer. Mix well.

8.7. Preparation for Extraction Recovery Control: Mix 20 µL of test sample solution with 20 µL of the 200 ng/mL standard solution to prepare the extraction recovery control solution.

8.8. Preparation for Negative Control Sample: Use the Sample Diluent Buffer directly for testing.

8.9. Plate Layout Example (Provide a plate layout as a reference, which can be adjusted as needed based on experimental requirements).

Plate Layout Example(Provide as a reference)

/	1	2	3	4	5	6	7	8	9	10	11	12
A	S0	S0	S#01	S#01	S#09	S#09	S#17	S#17	S#25	S#25	S#33	S#33
B	S7	S7	S#02	S#02	S#10	S#10	S#18	S#18	S#26	S#26	S#34	S#34
C	S6	S6	S#03	S#03	S#11	S#11	S#19	S#19	S#27	S#27	S#35	S#35
D	S5	S5	S#04	S#04	S#12	S#12	S#20	S#20	S#28	S#28	S#36	S#36
E	S4	S4	S#05	S#05	S#13	S#13	S#21	S#21	S#29	S#29	S#37	S#37
F	S3	S3	S#06	S#06	S#14	S#14	S#22	S#22	S#30	S#30	S#38	S#38
G	S2	S2	S#07	S#07	S#15	S#15	S#23	S#23	S#31	S#31	ERC	ERC
H	S1	S1	S#08	S#08	S#16	S#16	S#24	S#24	S#32	S#32	NC	NC

8.10. Sample Loading:

Placing the equilibrated Coated Plate in biosafety cabinet, add 50 µL of Virus Lysis to each well. Add 10 µL of the following to each well: HIV-1 p24 Standards: 0 ng/mL, 1.37 ng/mL, 4.12 ng/mL, 12.35 ng/mL, 37.04 ng/mL, 111.11 ng/mL, 333.33 ng/mL, 1000 ng/mL; Test samples; Negative control; Extraction Recovery Control; Internal control. With 2 replicate wells..

8.11. Addition of Enzyme-Labeled Secondary Antibody: Add 50 µL of Anti-HIV-1 p24+HRP to each well.

8.12. 1st Times Incubation: Seal the plate with plate-sealing film. Incubate in a constant temperature shaker at 18 ~ 25° C, 500 rpm, for 30 minutes.

8.13. Washing: In the biosafety cabinet, carefully remove the sealing film. Discard the liquid from the wells into a waste container. Tap the plate on absorbent paper to dry. Add 300 µL of 1× Wash Buffer to each well and let it sit for 30 seconds. Discard the liquid again and tap the plate dry. Repeat this process four times. Ensure no residual liquid remains in the wells.

8.14. Color Development: Add 100 µL of Color Reagent to each well. Perform this step in the biosafety cabinet , protected from light (turn off lighting).

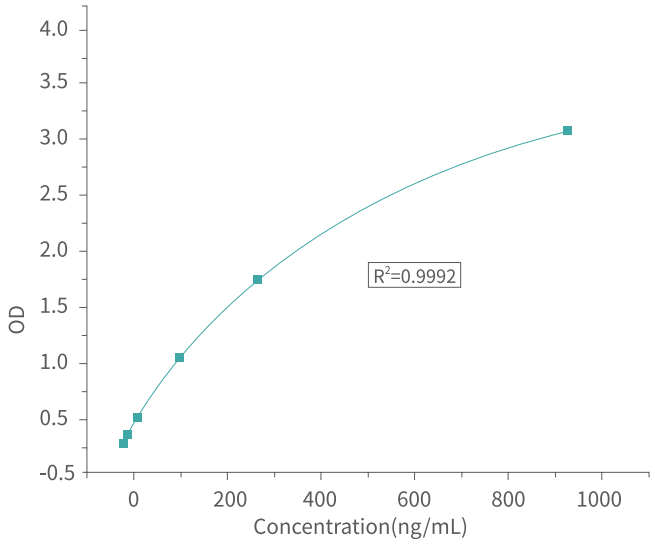
8.15. 2nd Times Incubation: Seal the plate and incubate it in a constant temperature shaker at 18 ~ 25° C, protected from light, for 5 ~ 10 minutes.

8.16. Reaction Termination: After the time is up, add 100 µL of Stop Solution to each well. Read the absorbance at a detection wavelength of 450 nm and a reference wavelength of 630 nm within 25 minutes using a microplate reader.

9. Results and Analysis

9.1. Standard Curve Fitting: Plot the absorbance of the standards on the y-axis and their concentrations on the x-axis. Use a four-parameter logistic regression equation to fit the standard curve.

9.2. Standard Curve Fitting Example Figure：



9.3. Calculation

9.3.1. p24 concentration in Test Sample (ng/mL):

p24=Dilution factor × p24 from 4 parameter fitting

9.3.2. Viral Physical Particle Number Count

Particle Number (PP/mL) = sample p24 centration (ng/mL) × 1.25 × 10^7

9.3.3. Internal Control Recovery Rate

$$\text{Recovery Rate} = \frac{\text{Internal Control Testing Concentration}}{\text{Internal Control Theoretical Concentration}} \times 100\%$$

9.3.4. Extraction Recovery Control (ERC)Recovery Rate

$$\text{Recovery Rate} = \frac{(C1 \times V3)-(C2 \times V2)}{C3 \times V1} \times 100\%$$

C1 = Extraction Recovery Control testing concentration

C2 = Sample Testing Concentration

C3 = Extraction Recovery Control Theoretical Concentration

V1 = Extraction Recovery Control Volume

V2 = Sample Testing Volume

V3= Total Volume (V1+V2)

9.4. System Suitability

9.4.1. For test values ⩾ 1.37 ng/mL (including standards, spiked QC samples, and test samples), the OD CV value for replicate wells must be ⩽ 20%.

9.4.2. R^2 of the standard curve should exceed 0.980.

9.4.3. Internal control recovery rate: 70% ~130%.

9.4.4. ERC Recovery Rate : 70% ~130%.

10. Troubleshooting

No.	Problem Description	Possible causes	Countermeasures
1	Standard curve gradient	Inaccurate pipetting or liquid dispensing	Check pipettes and tips
		Insufficient washing cycles of the microplate	Ensure the correct number of washing cycles and the appropriate volume of wash buffer per well
2	Both the standard curve and samples show no color development or very weak color development	Insufficient incubation time	Allow sufficient incubation time
		Incorrect experimental temperature	Use the recommended incubation temperature
		Omission of a critical component, especially detection antibodies or enzymes	Check experimental records and remaining reagents. Verify labels before each addition
		Expired Reagents	Use products within their expiration date
		Inactivation or loss of standards; antibodies; enzymes; substrates	Properly store and replace standards, antibodies, enzymes, and substrates as needed
		Delay in adding the next reaction solution after washing and drying the plate	Immediately add the next reaction solution after washing and drying the microplate
3	The standard curve shows no color development or very weak color development, but the samples develop color.	Insufficient vortexing when performing serial dilutions of standards	Use vortex mixing for dissolving and diluting
4	Low OD readings	Incorrect settings on the microplate reader	Verify wavelength and filter settings on the microplate reader
			Preheat the microplate reader before taking measurements
		Improper washing of the microplate: For example, excessive washing so long or prolonged standing of the washing solution in the wells.	Follow the washing protocol recommended in the manual
5	High coefficient of variation (CV)	Mismatch between pipettes and tips	Replace pipette tips
		Inaccuracy of pipette calibration	Regularly calibrate and test pipetting equipment
		Have residue or abnormalities on bottom of microplate	Check the bottom of the microplate for residual liquid or fingerprints and remove all of them
		Inconsistent pipetting	Practice pipetting to ensure consistent pipetting.
		Abnormalities in the wells	Confirm there are no foreign objects in the wells before adding samples and no air bubbles after

No.	Problem Description	Possible causes	Countermeasures
6	Detection interference value is too high	Insufficient washing of the microplate	Follow the manual's washing protocol recommendations
			If using an automatic plate washer, check all liquid inlet and waste outlet for blockages
			For manual washing, increase the number of wash cycles if necessary
		Contamination of reagents, such as ultrapure water	Replace contaminated reagents and re-prepare solutions
		Contamination of equipment, such as pipettes and centrifuges	Use dedicated pipettes and sterile, filter-equipped tips
		Unclean experimental environment, with ELISA test areas mixed with cell culture or lysis zones	Separate experimental operations into designated areas
		Incorrect reagent preparation, such as inappropriate dilution of washing buffer or detection antibodies	Re-prepare dilutions according to the correct dilution factor
		Excessively long reaction time	Use stop solution immediately when sufficient color development has been achieved, and reduce incubation time if needed
		Failure to protect the substrate incubation process from light	Substrate incubation should be performed in the dark
7	Experimental results deviate significantly from the reference parameters	Improper storage of the kit	Store reagents according to the manual's requirements
		Expired reagents.	Confirm the kit and its components are within their expiration date
		Failure to strictly follow the protocol during the experiment	Provide training to personnel before the experiment to ensure smooth execution
			Strictly control critical steps such as concentration, sample volume, and incubation time; do not substitute manual judgment for the instructions
8	Positive results observed in the negative control	Contamination of samples or reagents, or cross-contamination caused by improper pipetting and splashing between adjacent wells	Replace reagents and handle with care
		Insufficient washing of the microplate	Before washing, fully empty antibody solutions, then fill wells completely with wash buffer to ensure thorough washing
9	Significant differences in sample values calculated from different dilution gradients	Strong matrix effects in the samples	Choose dilution gradients where sample values are close, particularly for samples with high target protein content

11. 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

12. Notice to Buyer

Our products are for research use only. They must not be used for any other purposes, including but not limited to human use, therapeutic or diagnostic applications, or any commercial uses. Transfer, resale, modification for resale, or manufacturing of commercial products or services to third parties is prohibited without our consent.

Please also comply with any applicable license requirements as described on the product webpage at https://www.hillgene.com.

For more product, intellectual property, and usage restriction information, visit https://www.hillgene.com.

Attachment 1: Safety Precautions

● General Instructions

Improper use of this product may result in personal injury or damage to instruments or equipment. Ensure that all personnel using this product have received laboratory safety training and are familiar with the safety information in this document.

(1)Read and understand the user manual and safety information provided by the manufacturer before using instruments or equipment.

(2)Before handling chemicals, review the applicable Safety Data Sheets (SDS) and use appropriate personal protective equipment (gloves, protective clothing, goggles, etc.).

● Biological Hazards

(1)Biological samples, such as tissues, body fluids, infectious pathogens, and blood from humans or animals, may pose risks of transmitting infectious diseases. Perform all work in facilities equipped with appropriate safety equipment, such as biosafety cabinets.

(2)Individuals working with potentially biohazardous materials must be trained per local regulations and institutional requirements.

● Hazardous Waste

Waste generated by instruments may be hazardous. Follow the safety guidelines provided under "Biological Hazards."

Attachment 2: Related products (For more products, please consult Hillgene https://www.hillgene.com)

Category	Product Name	Cat. No.
Viral Detection	Human Residual DNA Detection Kit (qPCR)	HG-HD001
	Human Residual DNA Fragment Analysis Detection Kit (qPCR)	HG-HF001
	E.coli Residual DNA Detection Kit (qPCR)	HG-ED001
	Plasmid Residual DNA Detection Kit (qPCR)	HG-ZL001
	E1A&SV40LTA Residual DNA Detection Kit (Multiplex qPCR)	HG-EA001
	Benzonase Nuclease ELISA Detection Kit	HG-BE001
	BSA ELISA Detection Kit	HG-BS001
	Trypsin ELISA Detection Kit	HG-TR001
	PG13 Residual DNA Detection Kit (qPCR)	HG-PG001
	Host Cell Residual DNA Sample Preprocessing Kit (Magnetic Bead Method)	HG-CL100
	Blood/Tissue/Cell Genomic DNA Extraction Kit	HG-NA100
	293T HCP ELISA Detection Kit	HG-HCP001
Viral Packaging	Lentiviral Packaging	HG-HIV-CUL-001
	CD19 CAR-T Premade Lentivirus	HG-CT1901
	CD19 CAR-NK Premade 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