TECHNICAL MANUAL

Lentivirus Titer p24 PharmaGenie ELISA Kit

- **SKU CODE:** AEGE00002
- **SIZE:** 96T
- **DETECTION PRINCIPLE:** Sandwich
- **RUO:** Research-Use-Only

Revised: 08/2025

Lentivirus Titer p24 PharmaGenie ELISA Kit

Please read entire manual carefully before starting experiment.

Table of Contents

1. Key Features

Assay Range:

1.37 ng/mL -1000 ng/mL

Limit of quantification:

1.37 ng/mL

Limit of detection:

1 ng/mL

Sensitivity:

0.35 ng/mL

Detection Method:

Sandwich

Sample Type:

Compatible with diverse host cells (CHO, E. coli, Vero, Human, plasmids, SV40LTA & EIA). Works with varied sample types (liquid, dry powder) and pH ranges (6.0–8.0).

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit at as indicated in section 4. Validity for 12 months . Date of expiration is on the ELISA Box label.

3. Product Description

Lentiviral vector (LVV) production requires rigorous quality testing to ensure safety, consistency, and functionality. Critical quality attributes (CQAs) include lentiviral transduction titer, residual host cell DNA and proteins, and mycoplasma contamination. Among these, viral titer measurement is essential for confirming the vector's ability to infect target cells. Two complementary titers are evaluated: the physical titer, which reflects the total number of viral particles, and the transduction titer, which measures the number of infectious particles. Physical titer is commonly determined by detecting vector-specific components such as the p24 capsid protein using ELISA or by quantifying vector genome RNA copy number via qPCR. Together, these assessments ensure that lentiviral vectors meet stringent quality standards for research and clinical applications.

Lentivirus Titer p24 PharmaGenie ELISA 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.

4. Kit Contents

No	Component Name	Specification	Storage
1	Coated Plate	1 x 96 wells	2 ~ 8°C
2	Anti HIV-1 p24+HRP	6 mL	
3	HIV-1 p24 Standard	S1~S7,S0	
4	Virus Lysis	6 mL	
5	Sample Diluent Buffer	50 mL	
6	10×PBST Wash Buffer	50 mL	
7	Colour Reagent	12 mL	
8	Stop Solution	12 mL	
9	Plate Sealer	5	-
10	Technical Manual	1 copy	-

Additional materials required:

1. 37°C incubator.
2. Plate Reader with 450nm filter.
3. Precision pipettes and disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution.
6. Absorbent paper.

5. Precautions

1. Store all reagents according to the instructions on the product label. Before use, allow all reagents to equilibrate to room temperature.
2. Before opening the secondary packaging, bring the pre-coated strip plates to room temperature. Return any unused strips immediately to the original packaging and reseal tightly. Store unused plates at 4°C for up to one month. All other unused reagents should be properly sealed or covered.
3. The volumes of the standard, biotinylated antibody, and enzyme conjugate are small. Perform a quick centrifugation prior to use to ensure that any liquid adhering to the tube walls or caps collects at the bottom.
4. Always use disposable pipette tips during the assay to prevent cross-contamination.
5. Inspect all kit components before use. To ensure accurate results, mix thoroughly when preparing dilutions, loading samples, or adding stop solution.
6. During the washing steps, after removing Wash Buffer, tap the plate dry on clean absorbent paper until no residual droplets or watermarks are visible. Do not insert tissue directly into the wells.
7. The TMB substrate is photosensitive, protect it from prolonged light exposure. Avoid contact with metal surfaces, as this may interfere with the reaction.
8. This kit is intended for single use and should be used within its stated shelf life.

6. Sample Preparation

Due to the inherent variability of biological samples and the specific requirements of individual assays, users are advised to optimize protocols in accordance with their own experimental conditions. Samples may be tested directly with this ELISA or diluted as necessary, based on experimental objectives and the physicochemical characteristics of the sample matrix.

Note: *For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.*

7. Reagent Preparation

Note: *Turn on the UV lamp in the biosafety cabinet for 30 minutes.*

1. **Equilibrate Reagents:** 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.
2. **Preparation for Wash Buffer:** Dilute the 10x PBST Wash Buffer with deionized water to prepare a 1x Wash Buffer. Mix well.
3. **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.*
4. **Preparation for Standard Solution:** Dilute standards to achieve the concentrations from the table below. Use this to build standard curve:

HIV-1 p24 Standard	Standard concentration (µg/mL)
ST1	1000
ST2	333.33
ST3	111.11
ST4	37.04
ST5	12.35
ST6	4.12
ST7	1.37
0	0

5. **Prepare an internal control solution (200 ng/mL):** Add 20 µL of 1000 ng/mL standard (S1) to 80 µL of Sample Diluent Buffer. Mix well.
6. **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.
7. **Preparation for Negative Control Sample:** Use the Sample Diluent Buffer directly for testing.

8. Assay Procedure

1. **Setup Plate:** Before starting the assay, determine the layout of the microplate to include all required standards, blanks, and samples. Assign wells for the standard curve in duplicate, ensuring a full range of concentrations is represented. Include at least one blank well to serve as the zero reference. All samples should be loaded in duplicate to ensure accuracy and reproducibility. Prepare the plate according to the finalized layout to avoid cross-contamination and facilitate clear data interpretation.
2. **Standard, Samples and Controls Addition:** 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.
3. **Addition of Enzyme-Labelled Secondary Antibody:** Add 50 µL of Anti-HIV-1 p24+HRP to each well.
4. **First Incubation**: Seal the plate with plate-sealing film. Incubate in a constant temperature shaker at 18 ~ 25° C, 500 rpm, for 30 minutes.
5. **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 1x 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.

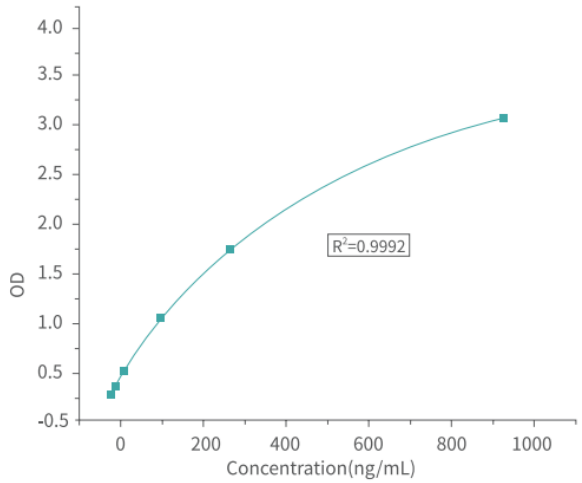
6. **Colour Development:** Add 100 µL of Colour Reagent to each well. Perform this step in the biosafety cabinet , protected from light (turn off lighting).
7. **Second Incubation:** Seal the plate and incubate it in a constant temperature shaker at 18 ~ 25 ° C, protected from light, for 5 ~ 10 minutes.
8. **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. Data Analysis

The 4-parameter fitting method is recommended for the linear fitting and calculation of the product.

OD processing of the standard curve (the following example is provided as reference only, and the results from actual detection shall prevail).

The standard curve is obtained by 4-parameter fitting with the theoretical standard concentrations and the corresponding OD values (as shown in the figure below).



The p24 concentration in the test sample (ng/mL) is determined by multiplying the dilution factor by the p24 value obtained from the four-parameter logistic (4PL) standard

curve fitting. The viral physical particle number (PP/mL) is then calculated by multiplying the sample p24 concentration (ng/mL) by 1.25×10^7.

The **Internal Control Recovery Rate** is calculated as:

$$Recovery\ Rate\ (\%) = \frac{Internal\ Control\ Testing\ Concentration}{Internal\ Control\ Theoretical\ Concentration}\ X\ 100$$

The **Extraction Recovery Control (ERC) Recovery Rate** is calculated using the following formula:

$$Recovery\ Rate\ (\%) = \frac{(CV1\ X\ V30 - (CV2\ X\ V2)}{C3\ X\ V1}\ X\ 100$$

Where:

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)

10. ELISA Troubleshooting

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect reagent order; Mixed components from different kits; Missing reagents.	Ensure correct reagent order and use components from the same kit. Verify all reagents are added.
Overflow OD	Mixed components from different kits; Over-concentrated working solution	Use correct components and prepare solutions at recommended concentrations.
Poor standard curve	Incorrect curve fitting model.	Try alternative curve fitting models.
Samples without signal	Sample concentration too low; Incompatible buffer; Incorrect preparation; Sample degradation or excessive freeze-thaw.	Reduce dilution or concentrate sample. Check buffer compatibility and follow proper preparation and storage.
High CV%	Precipitate formation; Unclean plate; Foaming; Uneven washing; Incomplete reagent mixing; Pipetting inconsistency.	Dilute samples if needed, avoid foaming, ensure uniform washing, mix reagents thoroughly, and use calibrated pipettes.
Low standard signal	Improperly reconstituted standards; Degraded standards; Incorrect pipetting; Expired kit; Improper storage; Over-dried wells.	Reconstitute standards properly, use fresh kits, follow storage recommendations, and prevent wells from drying.
Slow colour development	TMB not equilibrated; Incorrect microplate reader wavelength; Over-washing.	Pre-warm TMB (30 min at 37°C), confirm correct wavelength (450 nm), and follow recommended washing times.
High background	Insufficient washing; Contaminated wash buffer; Excess detection reagents; Delayed reading; TMB exposed to light.	Wash adequately, prepare fresh wash buffer, use correct reagent amounts, read results promptly, and incubate TMB in the dark.

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If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.