Lentivirus Titer p24 ELISA Kit
(One-step ELISA)
User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1703100

Version: A/0
For Research Use Only

Huzhou Shenke Biotechnology Co., Ltd.

■ Product Name

Lentivirus Titer p24 ELISA Kit (One-Step ELISA)

■ Package

96 tests/Kit

■ Intended Use

This kit is suitable for detection of lentiviral titer in HEK293T cell-derived virus production, amplification and purification.

The kit is for RESEARCH USE ONLY and not intended for clinical use.

■ Product Description

This kit is based on the solid-phase enzyme-linked immunosorbent assay (ELISA) with a double-antibody sandwich technique to detect Human Immunodeficiency Virus type 1 (HIV-1) p24/Capsid Protein p24. The 96-well strip plate is precoated with a monoclonal antibody specific to HIV-1 p24 / Capsid Protein p24. The Lysis solution, Calibration Standard (or test sample) and the HRP (Horseradish Peroxidase) labeled anti-p24 antibody were simultaneously added to the microtiter plate. Then TMB (3,3',5,5'-tetramethylbenzidine) substrate was added into the reaction, HRP catalyzed the oxidation of TMB by H_2O_2 to produce a blue product. Then the stop solution was added to terminate the enzymatic reaction, resulting in a yellow colored product (maximum absorption peak at 450 nm). The absorbance values at 450 nm wavelength were positively correlated with the p24 concentration in the Calibration standard and the sample. The concentration of p24 in the sample can be calculated using a dose-response curve.

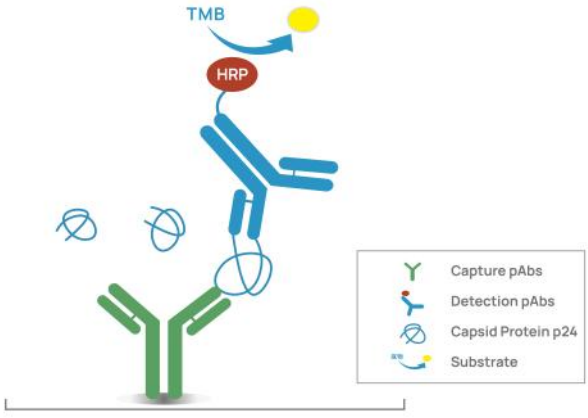


Figure 1. Schematic diagram

■ Kit Contents

Table 1.Kit Components

Reagent	Part No.	Quantity	Note
p24-Ag Calibration Standard	PNB014	1 × 300 μL	The solution should be transparent, without insoluble particles. Please refer to the details on the label of the tube.
Lysis Solution	PNS002	1 × 1.2 mL	The solution should be transparent, without insoluble particles.
Anti-p24 Microtiter Strips	PNA014	8 well × 12 strips	Strips pre-coated with HIV-1 p24 / Capsid Protein p24 monoclonal antibody in a vacuumed bag with desiccant. Seal and store immediately after use.
Diluent	PNE004	2 × 25 mL	For dilution of Calibration Standard, Anti-p24:HRP and samples.
Wash Buffer Concentrate (10×)	PNF001	2 × 25 mL	Dilute 10 times with freshly prepared ultra-pure water for plate washing.
Anti-p24:HRP (100×)	PNN008	1 × 120 μL	HIV-1 p24 / Capsid Protein p24 monoclonal antibody conjugated to HRP in a protein matrix with preservative. Dilute 100 times in Diluent (PNE004) before use.
TMB Substrate	PND005	1 × 12 mL	Sealed and keep away from light. Equilibrate to room temperature (RT) for 20 minutes before use.
Stop Solution	PNI002	1 × 6 mL	Avoid direct contact with eyes, skin, and clothing.
Sealing Film	PNK001	3 pieces	Cover the strips with it during incubation to prevent contamination and liquid evaporation.

Note: *Room temperature refers to 25±3℃.*

■ Storage Conditions

Store the kit at 2-8℃. Please check the expiration date on the labels. The opened components should be stored as follows.

Table 2. Recommended storage conditions for opened components

Component	Stability
Anti-p24 Microtiter Strips	Store in the vacuumed bag with desiccant at 2-8℃ for up to 30 days.

■ Materials Required But Not Provided

- Sterile centrifuge tubes for dilution
- Absorbent paper for plate drying
- Pipette Tips: 1000 μL, 100 μL and 10 μL
- Multi-channel reagent reservoirs (50 mL)

■ Equipment

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 620 nm to 650 nm.
- Single or multi-channel pipettes
- Microplate thermoshaker
- Incubator (optional)
- Plate washer (optional)

■ Workflow

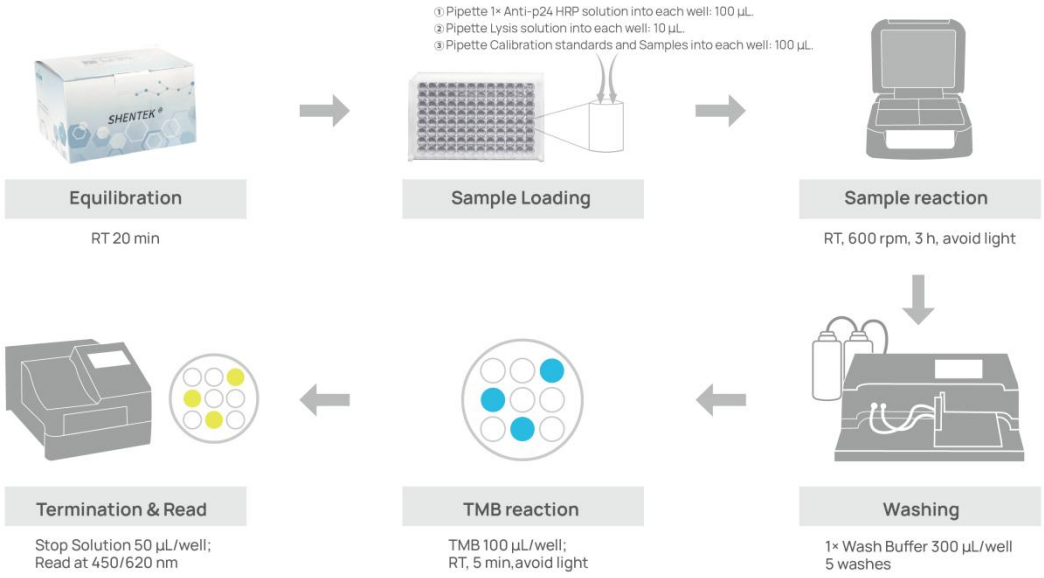


Figure 2. Procedure Flowchart

1. Preparation

(1) Equilibration

- Allow the kit to equilibrate at room temperature for 20 minutes before use. Return to 2-8℃ after use.
- Take the appropriate amount of strips to a strip holder according to the experiment design. Please store the remaining strips in the bag with desiccant at 2-8℃.

(2) Preparation of Reagents

- 1×Wash Buffer: Dilute 1 volume of Wash Buffer Concentrate (10×) in 9 volumes of ultra-pure water. For example, add 25mL Wash Buffer Concentrate (10×) to 225mL of ultra-pure water to make 250mL of 1×Wash Buffer. Prepare fresh and mix well before use.

  Note: *If the Wash Buffer Concentrate (10×) or Diluent is cloudy or contains precipitates, heat at 37℃ until it clear.*
- 1×Anti-p24:HRP: Prepare the 1×Anti-p24:HRP by diluting the Anti-p24:HRP (100×) with Diluent in a sterile centrifuge tube. Gently mix the solution and use it immediately.

(3) Preparation of Calibration Standard Solutions

- Prepare p24-Ag Calibration Standard Solutions as indicated in Figure 3 and Table 3.

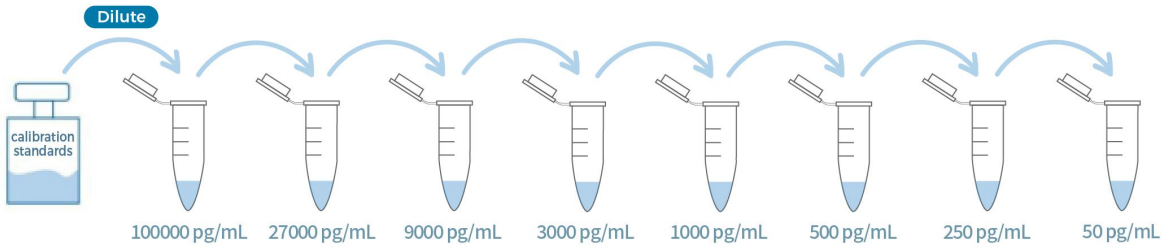


Figure 3.Graphic scheme of p24-Ag Calibration Standard Solutions

Table 3. Preparation of p24-Ag Calibration Standard Solutions

Tubes	Dilution procedure	Conc. (pg/mL)
ST0	Dilute the p24-Ag Calibration Standard to ST0	100000
ST1	270 µL ST0 + 730 µL Diluent	27000
ST2	300 µL ST1 + 600 µL Diluent	9000
ST3	300 µL ST2 + 600 µL Diluent	3000
ST4	300 µL ST3 + 600 µL Diluent	1000
ST5	400 µL ST4 + 400 µL Diluent	500
ST6	400 µL ST5 + 400 µL Diluent	250
ST7	100 µL ST6 + 400 µL Diluent	50
NCS	Diluent	0

(4) Sample Preparation

- Test samples: In-process samples, harvested bulk, Make sure samples are clear and transparent, and insoluble substances need to be removed by centrifugation or filtration.
- Conduct sample stability studies to prevent degradation or denaturation during the experiment. Long-term storage at -70°C is recommended to avoid degradation, and avoid repeated freeze-thaw cycles.
- Dilute the samples with a suitable diluent to achieve a proper range of HIV-1 p24 / Capsid Protein p24 concentration within the calibration curve.
- For the first use, a method validation is recommend to verify sample suitability before the subsequent routine test. This will help to set up appropriate sample

dilution series.

Note: *Please contact us for support of validation protocol.*

2. Assay Experiment

(1) Sample Loading

- Pipette 100 μL of 1×p24:HRP into each designated well according to the experimental design.
- Pipette 10 μL of Lysis Solution, 100 μL of Calibration Standard Solutions, NCS and samples into the corresponding wells as indicated earlier. Avoid foaming bubbles during pipetting. We recommend to prepare 2-3 replicates for each sample.
- Seal the plate and incubate on microplate thermoshaker at 600 rpm for 3 hours at room temperature and protect from light.

Table 4. Example of 96-well plate layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NCS	NCS	NCS		S1	S1	S1					
B	ST7	ST7	ST7		S2	S2	S2					
C	ST6	ST6	ST6		S3	S3	S3					
D	ST5	ST5	ST5		S1+SRC	S1+SRC	S1+SRC					
E	ST4	ST4	ST4		S2+SRC	S2+SRC	S2+SRC					
F	ST3	ST3	ST3		S3+SRC	S3+SRC	S3+SRC					
G	ST2	ST2	ST2									
H	ST1	ST1	ST1									

- ✧ “ST1-ST7” indicate 7 concentration gradients, “NCS” as negative control, “S1-S3” as test samples, and “S1 SRC-S3 SRC” as spiked recovery controls for each sample.
- ✧ The number of replicates and the inclusion of spiked samples can be determined based on the results of method validation

(2) Substrate Incubation

- Equilibrate the TMB Substrate for 20 minutes at room temperature.
- Wash the plate with 300 μL of 1×Wash Buffer per well. Wipe off any liquid from the bottom outside of the plate. Repeat washing for 5 times. Do not allow the

wells to be completely dry before adding the substrate.

- Add 100 μL of TMB Substrate into the wells, and incubate at RT for 5 minutes, protect from light.

  Note: *Do not use sealing film during this step.*

(3) Termination

- Add 50 μL of Stop Solution into each well.

  Note: *The order of adding stop solution should be the same as the order of adding the TMB Substrate. While adding samples, suspend the tips above the liquid to prevent contact with the solution in the wells and minimize the risk of bubble formation.*

(4) Reading

- Read absorbance at 450 nm/620-650 nm.

3. Calculation and Analysis

(1) Calculation

- The OD value of each well should be calculated by subtracting their respective long wavelength, as of $OD_{620\ nm}$ in this case. If the microplate reader is not equipped with long wavelength measurement, this step can be omitted.
- The $OD_{450\text{-}620\text{-}N}$ value of calibration curve fitting points and samples should be calculated by subtracting the $OD_{450\text{-}620}$ of NCS, then take the average value of replicates.
- Perform a 4-parameter logistic regression model using the subtracted Calibration Standard concentration values to obtain a calibration curve equation. The data analysis software for calibration curve fitting could be the built-in software from microplate reader. If not, we recommend to use professional standard curve software such as Curve Expert, ELISA Calc, and so on.
- Substitute the average OD value of the samples into the equation to calculate the sample concentration.

(2) Analysis

- For the sample with high OD value that exceeds ST1 concentration, a pilot study

should be performed to determine an appropriate dilution before retesting. The measured concentration of Human Immunodeficiency Virus type 1 (HIV-1) p24 / Capsid Protein p24 need to multiple by its corresponding dilution factor. If the spiked samples are simultaneously set at this dilution level and the recovery rate should meet the requirements of the corresponding regulations.

(3) Lentiviral Titer Calculation

- To calibrate the virus production system and establish a correlation between p24 level and infectivity, it is recommended to first determine the actual infectious titer (TU/mL) by functional methods such as expression of a fluorescent protein or drug-selective marker, and then relate it to the p24 content.
- The formula is calculated following the principles that each Lentivirus particle (LP) contains approximately 2000 molecules of p24.

  Normally, only one out of 100-1,000 LPs is infectious, *i.e.*, 1 TU (Transducing Unit) corresponds to 2×10^5-2×10^6 p24 protein molecules.

$$\text{Lentivirus Titer（TU/mL）} = \frac{\text{p24}(\text{g}\cdot\text{mL}^{-1}) \times 6.02 \times 10^{23}(\text{mol}^{-1})}{2.4 \times 10^4(\text{g}\cdot\text{mol}^{-1}) \times \text{a}\ (\text{TU}^{-1})}$$

  $a = 2 \times 10^5$–2×10^6 (dimensionless, molecules per TU).

  Note: *This lentiviral titer formula is for estimation only; actual functional titer prevails.*

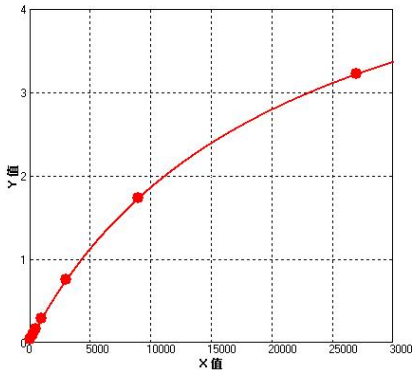
■ Limitations

- This product is intended for research use only but not for clinical use.
- The samples pH should be between 6.5 and 8.5. Beyond this range may cause abnormal results.

■ Assay Performance

- Linearity & Range: 50-27000 pg/mL，$R^2 \geq 0.990$
- QL: 50 pg/mL
- Specificity: No cross-reactivity with HEK293T HCP and 10% fetal bovine serum.
- Typical calibration curve and data for reference:

Calibration Standards (pg/mL)	Abs. At (450 nm-620 nm)	AVG
27000	3.2281	3.2199
	3.2249	
	3.2066	
9000	1.7480	1.7378
	1.7383	
	1.7270	
3000	0.7469	0.7513
	0.7603	
	0.7466	
1000	0.2885	0.2905
	0.2961	
	0.2870	
500	0.1589	0.1601
	0.1606	
	0.1609	
250	0.0921	0.0926
	0.0928	
	0.0929	
50	0.0390	0.0413
	0.0274	
	0.0274	
0	0.0265	0.0271
	0.0274	
	0.0274	



4-PL: $Y = \frac{A-D}{1+(\frac{X}{C})^B} + D$

A= 5.75850

B= -0.98459

C= 21369.92744

D= 0.02346

R^2= 1.00000

■ Additional Information

- ✧ This kit is intended for use by qualified technicians only.
- ✧ Consumables, for example sterile disposable tips, tubes and reservoirs are only allowed for single use. It is recommended to wipe with 75% ethanol before and after each use. Follow the specified pipetting procedure carefully.
- ✧ Dilution should be gentle and thorough to avoid excessive foaming.
- ✧ Stop Solution is 1M HCl. Avoid direct contact with eyes, skin, and clothing.
- ✧ Do not mix the kit reagents from different lot numbers.
- ✧ All biological samples pose potential safety risks. Please wear appropriate personal protective equipment during experimental operation, meanwhile dispose and discard the samples properly.
- ✧ Use fresh sterile water or ultra-pure water, and ensure the water temperature does not exceed 37℃.
- ✧ Add the sample to the bottom of the microplate, and try not to touch the wall. Pipette carefully to avoid any bubbles, and gently shake the plate for thorough mixing. Bubbles can influence optical density values and detection results. If there are bubbles before the plate reading, carefully remove the bubbles with a clean 10 μL pipette tips or needle.
- ✧ Seal or cover the microplate immediately after sample loading to avoid liquid evaporation.
- ✧ Avoid drying the wells before substrate incubation.
- ✧ Store unused microtiter strips in a sealed bag with desiccant to prevent contamination.
- ✧ Centrifuge Anti-p24:HRP(100×) before use to avoid any loss of the reagent.
- ✧ To avoid pipetting errors, pipette or sampling accurately for dilution of standards and samples, for example, a minimum volume of 5 μL is recommended.
- ✧ p24-Ag Calibration Standard Solutions and 1×Anti-p24:HRP are recommended for single use due to instability issue. Prepare freshly before each experiment.
- ✧ TMB Substrate should be colorless. If not, discard it and contact us for assistance.
- ✧ Read the plate immediately after termination.
- ✧ Avoid the samples containing sodium azide (NaN_3), which will deactivate the HRP and lead to the underestimation of HCP levels.

■ Troubleshooting

Problem	Possible Cause	Solution
High background signal (OD)	Cross-contamination of reagents, including distilled water	Freshly prepared prior to experiment.
	Cross-contamination of equipment, including micropipettes and centrifuge	Clean the equipment with 75% ethanol before experiment.
	Environment contamination	Separate the working bench to avoid contamination.
	Insufficient washing	Increase the wash buffer volume or wash times, and remove any remaining liquid before proceeding to the next step.
Abnormal values	Improper washing	Swiftly and completely shake off any excess liquid, and avoid reusing paper towels to minimize contamination.
	Improper sampling	Add the samples to the bottom of the wells using pipettes, and avoid splashing to the neighboring wells.
	Plate sealing	Promptly cover the plate with the sealing film and remove it carefully to prevent splashing.

If you have any other questions, please contact us for technical support.

■ References

- USP <1103> Immunological Test Methods - Enzyme-Linked Immunosorbent Assay (ELISA)
- ICH. M10 Bioanalytical Method Validation And Study Sample Analysis
- JP <G3-11-171> Enzyme-Linked Immunosorbent Assay (ELISA)
- White S M, Renda M, Nam N Y, et al. Lentivirus vectors using human and simian immunodeficiency virus elements. Journal of virology, 1999, 73(4): 2832-2840.
- Kahl C A, Marsh J, Fyffe J, et al. Human immunodeficiency virus type 1-derived lentivirus vectors pseudotyped with envelope glycoproteins derived from Ross River virus and Semliki Forst virus. Journal of virology, 2004, 78(3): 1421-1430.

Effective date: 19 Dec. 2025

Support & Contact

Huzhou Shenke Biotechnology Co., Ltd.
www.shentekbio.com
Address: 8th Floor, 6B Building, No.1366 Hongfeng Road, Huzhou 313000, Zhejiang Province, China
E-mail: info@shentekbio.com
Phone: +1 (908) 822-3199 / (+86) 400-878-2189