EZElisa™ HIV Lentivirus/HIV-1 P24 Titer ELISA Kit

Cat #: A-QEK009

Size: 96 Tests

Storage: 2-8 °C in dark (The production date and shelf life are shown on label of the kit.)

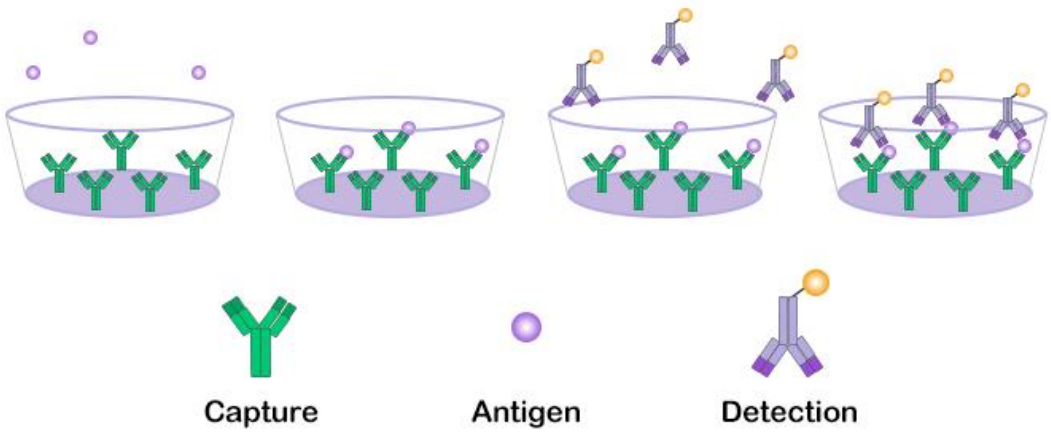
Product information

Product description

The kit for the detection of lentivirus titer (HIV-1 P24) produced by our company is a specialized kit for qualitative and quantitative detection of HIV-1 P24 protein using the enzyme-linked immunosorbent assay (ELISA) method. This kit is easy to use, exhibits good reproducibility, and has high stability. It is suitable for the titration detection of lentivirus in serum, culture supernatant, and other samples, enabling users to achieve efficient lentivirus titer quality control.

Principles

This kit utilizes the sandwich enzyme-linked immunosorbent assay (ELISA) technique with dual antibodies to detect HIV-1 p24 protein. The capture antibody is coated on a 96-well microplate to form a solid-phase antibody. Subsequently, standard samples and test samples are added in the presence of a lysis buffer, followed by the addition of a detection antibody labeled with horseradish peroxidase (HRP). This forms a sandwich complex of solid-phase antibody-p24 protein-labeled antibody. After the reaction is complete, the plate is washed, and a substrate is added for color development. The substrate is converted to blue under the catalysis of HRP and then converted to the final yellow color under the action of a stop solution (as shown in the figure below). The absorbance (OD value) is measured at 450nm and 630nm wavelengths, with 630nm as the correction wavelength. The content of p24 protein in the test sample is calculated using the standard curve, and the p24 content correlates with the viral titer in the packaging cell supernatant.



Performance specifications

Detection Limit: < 0.02 ng/mL

Quantification Limit: 0.0617 ng/mL

Linear Range: 0.0617 - 15 ng/mL

Accuracy (Recovery Rate): 70% - 130%

Accuracy (Measurement Deviation): ≤ 15%

Reproducibility (Within-Batch Variation): ≤ 10%

Materials provided

Kit components	Size (96 Tests)	Storage conditions
Microplate	8 × 12	2-8 °C
Lysis buffer	1.5 mL × 1	2-8 °C
Standard solution (150 ng/mL)	1 mL × 1	2-8 °C
Enzyme-labeled antibody (100×)	150 µL × 1	2-8 °C
Sample diluent	60 mL × 1	2-8 °C
Wash Buffer (20x)	30 mL × 1	2-8 °C
Developing solution A	8 mL × 1	2-8 °C protected from light
Developing solution B	8 mL × 1	2-8 °C protected from light
Stop solution	15 mL × 1	2-8 °C
Microplate Sealers	3	

Note: The kit should be stored light-protected at 2-8°C. After opening, any unused reagents should still be stored light-protected at 2-8°C.

Please refer to the kit label for the production date and expiration date.

Procedure

Reagents/Equipments required but not supplied

Allow the kit components to equilibrate at room temperature for 30 minutes before starting the procedure.

1. Required Consumables and Equipment: Microplate reader, shaking incubator (or incubator), plate washer (hand washing is acceptable), vortex mixer, timer.
2. High-precision pipettes and disposable tips (0.5-10 µL, 10-100 µL, 30-300 µL, 100-1000 µL).
3. Deionized water.
4. Absorbent paper, EP tubes, disposable gloves.

Reagent preparation

1. Wash solution (1×): Prepare by diluting the wash solution (20×) with deionized water at a 20-fold dilution, for example: mix 10 mL of the wash solution (20×) with 190 mL of deionized water.

Note: If crystallization occurs in the wash solution (20×), gently shake it at room temperature or in a 37 °C water bath until the crystals are completely dissolved before dilution.

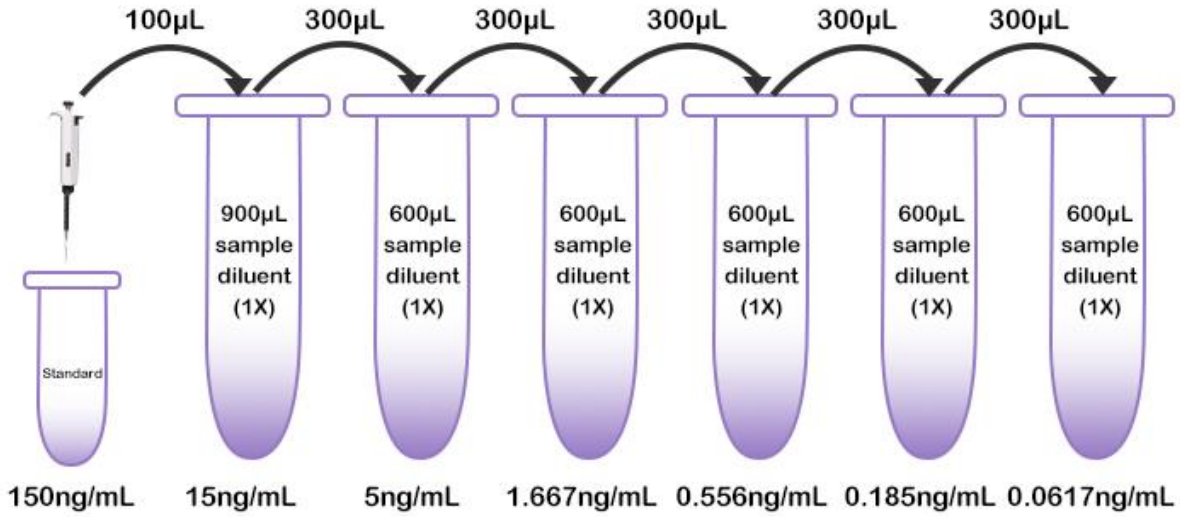
2. Preparation of color development solution: Mix the same volumes of Developing solution A and Developing solution B, mix well, and keep protected from light.

Note: Do not prepare the solution too far in advance, generally prepare it within 10 minutes before use. If the mixed substrate solution has turned blue, do not use it.

3. Preparation of enzyme-labeled antibody solution: Dilute the enzyme-labeled antibody (100×) with the dilution buffer at a 100-fold dilution to obtain the enzyme-labeled antibody (1×) solution. For example, add 10 µL of the enzyme-labeled antibody (100×) to 990 µL of sample dilution buffer.

4. Preparation of standard solution (**Note: Prepare a fresh standard solution for each experiment**)

Dilute the standard to 15 ng/mL using the sample diluent, then prepare the standard solution by further diluting it in a 3-fold serial dilution method, as shown in the figure below.



Sample preparation

1. For unknown samples, especially those with very high p24 titers (i.e., >15ng/mL or 1.87E+08 LP/mL), they must be diluted before testing to obtain accurate p24 titer values within the linear range of the kit. We recommend performing multiple consecutive 10-fold dilutions of the unknown samples to ensure that at least one diluted sample falls within the range of the standard curve. Typically, samples need to be diluted 10-1000 times.

2. It is recommended to prepare duplicate of all samples. Multiply the results by the dilution factor to determine the p24 value in the original sample, as described in the calculation of lentivirus titer.

Assay Procedure

All operations are performed at room temperature, and it is recommended to perform duplicate well measurements for both standard and sample.

1. Allow all components of the kit to equilibrate at room temperature for 30 minutes. Take out the required microplate strips from the foil pouch that has been equilibrated to room temperature, mark the order of the strips with a marker pen, seal the remaining strips with a sealing film, and place them back into the foil pouch. Store at 2-8 °C.

Note: The strips are prone to fall off during the washing process, so be sure to make proper markings.

2. Add 10 µL of lysis buffer to each reaction well.

3. Sample incubation: Add the diluted standard and test samples to the microplate (recommended order: standard wells, blank wells, sample wells; add the standard in concentration gradient), 90 µL/well. Seal the plate with a sealing film, shake for 30-60 seconds to mix well, and then incubate at 37 °C for 30 minutes.

Note: Control the sample addition time within 10 minutes to avoid drift over time. If the plate is not sealed or the sealing is incomplete during incubation, the evaporation of the reaction solution may lead to experimental errors.

4. Wash the plate: After incubation, carefully remove the sealing film, discard the liquid in the wells, wash the plate three times with wash solution (1×) (250 µL/well), and tap to remove residual liquid in the sample wells.

Note: If hand washing is used, let the wash solution (1×) sit for 1 minute; if using a plate washer, gently shake for 5 seconds after adding the wash solution.

5. Enzyme-labeled antibody incubation: Add the enzyme-labeled antibody to each well, 100 µL/well. Seal the plate with a sealing film and incubate at 37 °C for 30 minutes.

Note: Before adding the liquid after each washing step, check if the strips are securely fixed to prevent liquid splashing out after fixing the strips.

6. Wash the plate: Same as step 4).

7. Color development: Add the pre-prepared color development solution to the microplate, 100 µL/well. Seal the plate with a sealing film and incubate at 37 °C in the dark for 15 minutes.

8. Termination: Add the stop solution, 100 µL/well. After uniform color development, the plate can be read (usually within 20 minutes after adding the stop solution).

Note: The enzyme immunoassay instrument can be set to shake for 5-10 seconds.

9. Reading: Place the microplate in the enzyme immunoassay instrument, set the wavelength to dual wavelengths of 450/630 nm, and read the absorbance values.

Calculation of results

1. Calculation of Absorbance Values

The calibrated absorbance value for each standard or sample is calculated as follows:

OD_{450nm} - OD_{630nm} - absorbance value of the blank control well

2. Plotting the Standard Curve

Using the concentration of the standard as the X-axis and the calibrated absorbance value of the standard as the Y-axis, plot the standard curve. It is recommended to use the four-parameter logistic mathematical model for curve fitting with the equation:

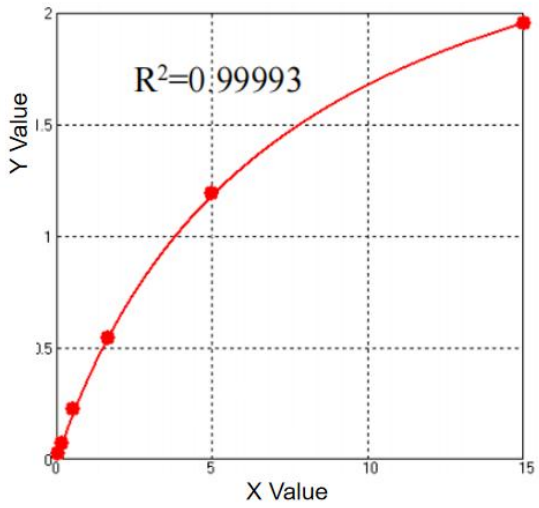
$$Y = (A - D) / (1 + (X / C) ^ B) + D$$

Substitute the sample's OD value (OD_{450nm} - OD_{630nm} - absorbance value of the blank control well) into the equation to calculate the p24 protein content in the sample, taking into account the dilution factor.

3. If the OD value of a test sample exceeds the highest point on the standard curve, the sample needs to be diluted and retested.

Example Display

The following standard curve graph is for reference only. The actual standard curve should be based on the standard curve plotted using the standards from the same experiment.



Standard curve (ng/mL)	OD Value
15.0000	1.9532
5.0000	1.1876
1.6667	0.5408
0.5556	0.2207
0.1852	0.0746
0.0617	0.0249

Virus Titer Calculation

The p24 content can be used to determine the relative virus titer in the supernatant of packaging cells. To calibrate the virus production system and establish the relationship between p24 levels and viral infectivity, it may be necessary to use other methods to determine the p24 levels in supernatants that have been previously measured for virus titers.

p24 concentration = p24 (ng/mL) × dilution factor

The following calculations can be used to determine an approximate titer. Each lentiviral particle (LP) contains approximately 2000 p24 protein molecules:

$$\text{One LP} = 2000 \times 24 \times 10^{3} / (6 \times 10^{23}) \text{ g of p24} = 8 \times 10^{-5} \text{ pg of p24}$$

$$1 \text{ ng of p24} = 1.25 \times 10^{7} \text{ LPs} \approx 1.25 \times 10^{4\text{-}5} \text{ TU}$$

Note: 1 infectious virus particle (1 TU) is present in every 100-1000 LPs. For example, a titer of 10^{7} TU/mL ≈ 10^{9}-10^{10} LP/mL or 80-800 ng p24/mL

Precautions

1. All components of the kit must be equilibrated to room temperature (20-25 °C) before use.

2. Thoroughly mix all components of the kit before use. When drying the plate after washing, be careful to prevent strip detachment.

3. The kit must be used within its expiration date, and it is not recommended to mix reagents from different batches. Do not mix with components from other manufacturers.

4. Strict adherence to the instructions and the use of all reagents provided in this kit are necessary to ensure optimal detection results.

5. P24 values obtained from different manufacturers or different methods should not be used interchangeably.

6. This kit cannot accurately determine the HIV-1 p24 protein content/virus titer in samples that exceed the linear range of the standard curve of the kit. Samples need to be appropriately diluted to ensure that the HIV-1 p24 protein in the sample falls within the linear range of the kit.

7. Pay attention to timely replacement of sample wells and tips between different samples and steps to avoid cross-contamination.

8. The final experimental results are closely related to the effectiveness of the reagents, the operator's techniques, and the experimental environment.

9. Our company is only responsible for the kit itself and is not responsible for the sample consumption caused by the use of the kit. Users should consider the potential sample usage and reserve an adequate amount of samples before use.

10. This kit is for in vitro research use only and is not intended for clinical diagnosis.

Safety Tips

1. VSV-G-pseudotyped lentiviral vectors (VSV-G-LV) derived from HIV-1 vectors can infect human cells. The viral supernatant produced by these lentiviral systems contains potentially hazardous recombinant viruses. For all materials containing BSL-2 organisms, please follow the guidelines recommended by the National Institutes of Health (NIH).

2. Any materials that may be contaminated with potentially infectious materials should be treated as infectious materials according to local regulations. For more detailed information, please contact the local laboratory safety committee.

3. All personnel handling lentiviruses must receive appropriate biosafety training.
4. The termination solution in this kit is an acidic solution, so extra caution should be taken during handling.
5. All biological samples carry potential biohazard risks. Users should strictly follow local laws and regulations for the handling and disposal of samples.
6. For safety considerations, operators should wear personal protective equipment such as lab coats, gloves, masks, and goggles.

Common Problems and Analysis

If there are issues with the experimental results, promptly take photos of the color development results and properly store unused strips and reagents. Then, contact technical support for assistance. You can also refer to the following information to identify the possible causes.

Problem description	Possible reasons	Corresponding countermeasures
Gradient bad in the standard curve	Incorrect dilution	Failure to dilute the standard curve according to the specified dilution factor
	Inaccurate liquid aspiration or addition	Check the pipette and tips
	Incomplete enzymatic plate washing	Ensure the appropriate frequency of plate washing and the volume of wash buffer per well
Weak or colorless color development	Insufficient incubation time	Ensure an adequate incubation time
	Incorrect experimental temperature	Use the recommended incubation temperature
	Insufficient or missed addition of reagents	Check the liquid aspiration and addition process to ensure all reagents are added in the correct order and in sufficient amounts
	Incorrect preparation of the color development solution	Mix equal volumes of color development solution A and B without exposure to light, prepare 10 minutes before color development
Low OD (Optical Density) reading	Incorrect settings on the plate reader	Check the wavelength and filter device on the plate reader
		Preheat the plate reader in advance before taking readings
High coefficient of variation (CV) value	Incorrect liquid addition	Check the liquid addition process
	Contamination on the bottom of the microplate	Check for residual liquid and fingerprints on the bottom of the microplate
	Foreign particles or bubbles in the wells	Ensure there are no foreign particles in the wells before sample addition and confirm the absence of bubbles after sample addition
	Unsealed or incomplete sealing of the microplate during incubation	Seal the microplate with a sealing film
High background value	Incomplete enzymatic plate washing	Follow the recommended washing method in the instructions.

		If using an automated plate washer, check for blockages in all liquid addition and waste disposal ports
		If washing manually, increase the number of washes appropriately
		Insufficient washing or incomplete washing can lead to high background
	Incubation time and temperature	Strictly follow the instructions for the recommended incubation conditions
	Contamination of consumables	Ensure the cleanliness of the tubes, tips, and other consumables used
	Contamination in the washing solution	Prepare fresh washing solution
	Contamination of the color development solution	The color development solution itself should be colorless. Ensure that the color development solution is not contaminated with metal ions or oxidizing reagents before use and store it in a light-protected manner
Low sensitivity	Improper storage of the kit	Store the relevant reagents according to the instructions

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.