HIV-p24 ELISA Kit

Please read the manual carefully before use.

Cat. No. NE107

Storage: at 2~8°C in the dark for one year.

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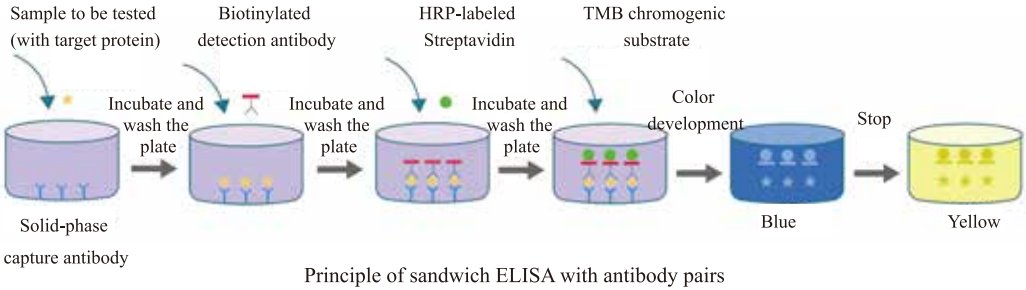
Description

p24 is a highly conserved and most abundant major structural protein in HIV virions. It is a product encoded by the structural gene gag and plays a key role in the packaging and maturation of the virus. Lentiviral vector is a viral vector system modified on the basis of HIV-1 virus, which can efficiently transfer the target gene into cells. The content of p24 protein represents the number of lentivirus particles, therefore, the physical titer of lentivirus can be determined by assaying the p24 content by ELISA method.

This kit uses Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) to measure the amount of HIV-p24 in lentiviral supernatant. The ELISA microplate in the kit is precoated with a high-affinity anti-human HIV-p24 antibody. The virus lysate and standard or test sample are added to the microplate wells. After incubation, HIV-p24 present in the sample will be bound specifically to the precoated antibody on the microplate wells. After washing, biotin-labeled anti-human HIV-p24 detection antibody is added to the microplate wells. After re-incubation, the detection antibody will be bound specifically to HIV-p24 anchored on the microplate wells. Subsequently, the horseradish peroxidase-labeled streptavidin (Streptavidin-HRP) is added to the microplate wells and incubated. Biotin and streptavidin on the detection antibody produces the "coated antibody-HIV-p24 protein-detection antibody-Streptavidin-HRP" immune complex through high-strength non-covalent binding. After washing again, the chromogenic substrate TMB is added to the wells. HRP catalyzes the TMB substrate to create blue coloration, with intensity positively correlated with the concentration of HIV-p24 in the sample. The reaction is stopped by adding stop solution, and the absorbance is measured at 450 nm (reference wavelength 570-630 nm). A standard curve is drawn to calculate the concentration of HIV-p24 in the sample based on the absorbance value. This kit is highly specific, has high detection sensitivity and is convenient to use.



Principle of sandwich ELISA with antibody pairs

Suitable Sample Types

Lentiviral supernatant.

Kit Contents

Component	NE107-01	Storage
HIV-p24 Antibody Precoated ELISA Plate	96 T	at 2~8°C
HIV-p24 Standard	120 µl/ bottle	at 2~8°C
Lysate	6 ml/bottle	at 2~8°C
1×HIV-p24 Detection Antibody	12 ml/bottle	at 2~8°C
1×Streptavidin-HRP	12 ml/bottle	at 2~8°C in the dark
20× Wash Buffer	50 ml/bottle	at 2~8°C
TMB Chromogenic Substrate	12 ml/bottle	at 2~8°C in the dark
Stop Solution	12 ml/bottle	at 2~8°C
Plate Sealers	4 pieces	

Note: The kit can be stored at 2~8°C for 1 month when it was opened; please use the unopened kit within 1 year.

Self-prepared Regents and Equipment

1. Deionized water.
2. Fresh complete medium (such as DMEM with 10% FBS).
3. EP tubes, pipettes, tips, graduated cylinders, etc.
4. Microplate shaker.
5. Automatic plate washer or 8-channel manual plate washeror multi-channel pipette.
6. Microplate reader: at a main wavelength of 450 nm, a reference wavelength of 620 nm.

Sample Collection

Lentiviral supernatant: Collect lentiviral supernatant and centrifuge it at 300×g, 4°C for 10 minutes. Aliquot the supernatant into EP tubes and store at -20°C. Avoid repeated freeze-thaw cycles. For detection within 24 hours, it can be stored at 2~8°C.

Sample Dilution

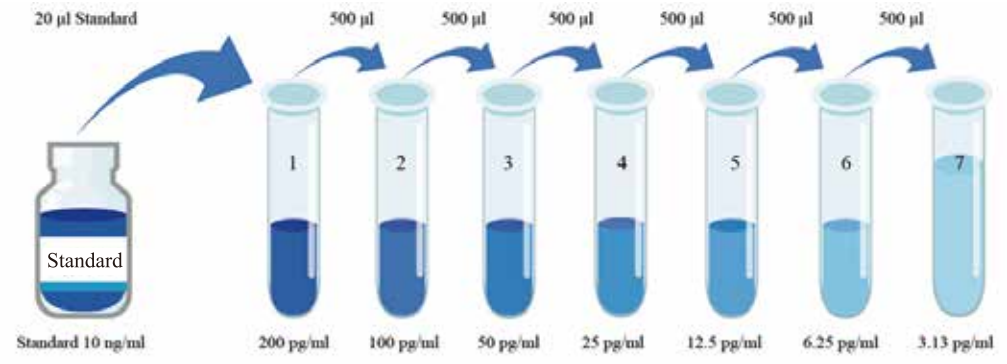
1. If the positive value of the Lentiviral supernatant sample is within the range of the curve, it does not need to be diluted with the fresh complete medium (such as DMEM with 10% FBS), and can be directly detected with the original solution; if the positive value exceeds the range of the curve, it needs to be diluted to the curve range with the fresh complete medium (such as DMEM with 10% FBS) for detection. Usually unconcentrated lentiviral supernatant needs to be diluted 10-10000 times. The concentration should be multiplied by the corresponding dilution factor.
2. **It is recommended to perform a pre-experiment to determine the dilution factor before the formal experiment.**

Working Solution Preparation

Bring all reagents to room temperature before use.

1. **1× Wash Buffer**: Dilute the 20× washing solution to 1× working concentration with deionized water according to the amount required for the current experiment. After preparation, it can be stored at 2~8°C for 30 days.
2. **Standard Gradient Dilution**: Dilute the standard（10 ng/ml）50 times with fresh complete medium (such as DMEM with 10% FBS), take 20 µl of the standard, add it to 980 µl fresh complete medium, mix well, record it as a tube 1, and the concentration of tube 1 at this time is 200 pg/ml; then perform a 2-fold gradient dilution according to the figure below, and add 500 µl of fresh complete medium to tubes 2-7 respectively; take 500 µl of the liquid from the No. 1 tube and add it to the tube 2. At this time, the concentration of the tube 2 is 100 pg/ml.

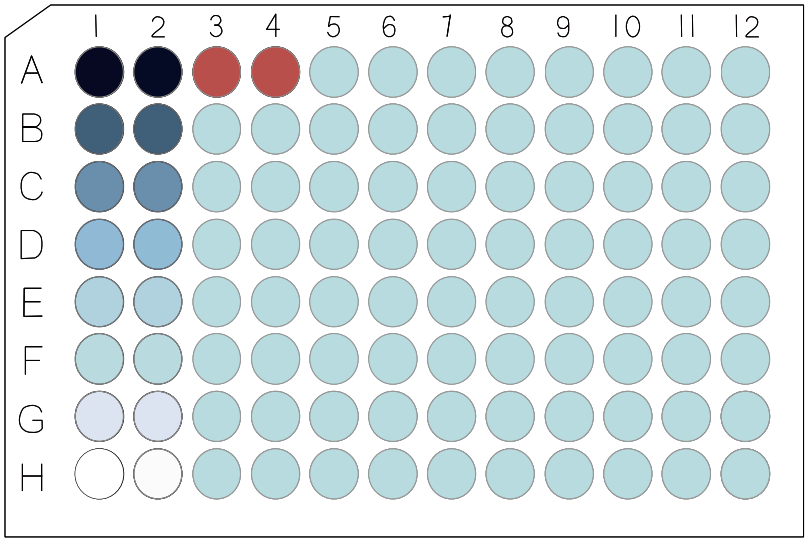
After mixing, take 500 µl and add it to the tube 3. At this time, the concentration of the tube 3 is 50 pg/ml; and so on for gradient dilution to tube 7 (3.13 pg/ml). 200 pg/ml as the highest point of the standard curve, fresh complete medium as the zero point (0 pg/ml) of the standard curve, that is, the blank value.



Experimental Protocol

1. Bring all reagents to room temperature before use. Take out the plates required for the current experiment, put the unused plates in an aluminium foil pouch in time to reseal, and store them at 2~8°C.
2. Add the lysate to wells of the plate, 20 µl/well, then add the diluted standard and sample to the wells of the plate respectively, 200 µl/well, shake on a microplate shaker for 30 seconds to mix well, cover the plate and incubate for 1 hours at room temperature. It is recommended that the standards and samples be tested in duplicate, and the order of adding reagents should be consistent so that the results of each duplicate well test are consistent.
3. Discard the liquid in the wells and wash wells the plate with 1× washing solution, 350 µl/well. It is recommended to shake the plate on a microplate shaker for 30 seconds before washing, and then discard the washing solution. Repeat 5 times, the last time pat dry on a paper towel.
4. Add 1× Detection Antibody to each well, 100 µl/well, shake on a microplate shaker for 30 seconds to mix well, cover the plate with a sealing film, and incubate for 1 hour at room temperature.
5. Repeat Step 3.
6. Add 1× Streptavidin-HRP to each well, 100 µl/well, shake on a microplate shaker for 30 seconds to mix well, cover the plate with sealing film, and incubate for 30 minutes at room temperature .
7. Repeat Step 3.
8. Add TMB Chromogenic Substrate to each well, 100 µl/well, shake on a microplate shaker for 30 seconds to mix well, cover the plate with a sealing film, and incubate for 15 minutes at room temperature.
9. After the incubation, add stop solution, 100 µl/well, and read absorbance of the plate at the wavelength of 450 nm for the main wavelength and 620 nm for the reference wavelength.
10. After the experiment, put the unused reagents and the outer frame of the ELISA plate back into the kit and store them at 2~8°C. It is recommended to use up within 1 month.

Sample Loading on The Microtiter Plate



Notes: A1/A2: 20 µl lysate + 200 µl 200 pg/ml standard
B1/B2: 20 µl lysate + 200 µl 100 pg/ml standard
C1/C2: 20 µl lysate + 200 µl 50 pg/ml standard
D1/D2: 20 µl lysate + 200 µl 25 pg/ml standard
E1/E2: 20 µl lysate + 200 µl 12.5 pg/ml standard
F1/F2: 20 µl lysate + 200 µl 6.25 pg/ml standard
G1/G2: 20 µl lysate + 200 µl 3.13 pg/ml standard
H1/H2: 20 µl lysate + 200 µl 0 pg/ml standard(fresh complete medium)
A3/A4: 20 µl lysate + 200 µl Lentiviral supernatant

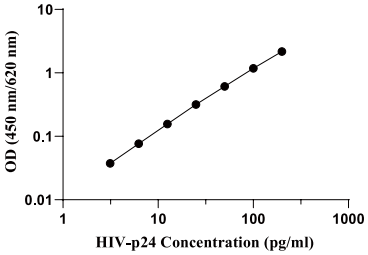
Result Analysis

1. Double-wavelength detection was performed with a microplate reader, and the OD values at the main wavelength of 450 nm and the reference wavelength of 620 nm were determined. The OD value is the OD measurement at 450 nm minus the OD measurement at 620 nm.
2. Calculate the average OD value of duplicate wells of the standard, then subtract the blank value (average OD value of the 0 pg/ml standard) to obtain the corrected value for the standard. Taking the concentration of the standard product as the abscissa and the OD correction value as the ordinate, the standard curve was generated by linear regression or the four-parameter method.
3. Calculate the sample concentration from the sample OD value and the standard curve equation. If the OD value of the sample is higher than the upper limit of the standard curve, it should be re-measured after appropriate dilution, and the corresponding dilution should be multiplied when calculating the concentration.

Reference Data

A standard curve needs to be established with each assay, and the following data are only for demonstration purposes.

Standard (pg/ml)	OD Value		Average	Corrected
200	2.159	2.203	2.181	2.170
100	1.215	1.159	1.187	1.176
50	0.630	0.617	0.6235	0.6125
25	0.331	0.326	0.3285	0.3175
12.5	0.167	0.167	0.167	0.156
6.25	0.087	0.088	0.0875	0.0765
3.13	0.048	0.049	0.0485	0.0375
0	0.011	0.011	0.011	0.000



Interpretation of the results of the standard duplicate wells: In the above table, the average OD value of the two duplicate wells of the 0 pg/ml standard is (0.011+0.011)/2=0.011, and the correction value is set as 0. The average OD value of the two duplicate wells of the 200 pg/ml standard is (2.159+2.203)/2=2.181, and the corrected value is 2.181-0.011=2.170.

Precision

Intra-assay Precision

Intra-assay precision was assessed by assaying 20 replicate wells on one microplate using 3 samples of known concentration.

Inter-assay Precision

Inter-assay precision was assessed by assaying 20 replicate wells on different microplates with 3 samples of known concentration.

	Intra-assay			Inter-assay		
	1	2	3	1	2	3
Average (pg/ml)	96.2	48.3	22.2	93.6	47.7	21.5
Standard Deviation	2.6	1.1	0.98	4.0	2.9	1.4
Coefficient of Variation (%)	2.7	2.3	4.4	4.3	6.1	6.5

Calibration

The standard of this kit is high-purity recombinant HIV-p24 calibrated by TransGen Biotech.

Sensitivity

The lowest detectable concentration of HIV-p24 was 1.0 pg/ml. Sensitivity is the corresponding concentration calculated from the mean of 20 replicate zero standard OD values plus two standard deviations.

Virus Titer

Calculate the approximate lentiviral titer of the viral supernatant from the data below.

Each lentiviral particle has approximately 2000 p24 molecules, so 1 viral particle contains 8×10^{-5} pg p24 (algorithm $2000 \times (24 \times 10^3) / (6 \times 10^{23}) \times 10^{12}$)

1 ng p24 is equivalent to 1.25×10^7 virus particles

Normally, 1 TU (Transducing Unit) contains about 100~1000 virus particles, so 10^7 TU/ml ≈ 10^9~10^{10} virus particles/ml ≈ 80~800 ng p24/ml.

Notes

1. This kit should be stored at 2~8°C in the dark and used up within 1 month after opening.
2. To ensure accurate results, a standard curve is required for each assay..
3. All reagents used in the experiment should be thoroughly mixed.
4. Add the lysate first, then add the standard/sample, and shake for 30 seconds to make it completely mixed.
5. After each plate washing, pat dry on a paper towel. If there are air bubbles in the plate wells, use a pipette tip to puncture them. Note that only one pipette tip can be used in each well to avoid cross-contamination.
6. TMB chromogenic substrate is a colorless and transparent liquid, please do not use it if there is discoloration.
7. After TMB develops color, it can be judged whether it is necessary to add a stop solution in advance or later according to the depth of color development.
8. After adding the stop solution, read within 30 minutes.
9. It is recommended to use the main wavelength of 450 nm and the reference wavelength of 620 nm for reading. If only a single wavelength of 450 nm is used for reading, the overall OD value may be high, and the blank value will also increase accordingly, resulting in a decrease in the accuracy of the kit.
10. Personal protective equipments are necessary in experiments for safety reasons. The stop solution in the kit is corrosive. Take care when using the reagent to avoid the risks. In case of accidental contact, please rinse with plenty of water and seek medical attention in time.
11. To avoid cross-contamination, use a new disposable pipette tips for each transfer. Please use disposable test tubes, pipette tips, plate sealers and clean plastic containers in the experiment.
12. Kit components from different batches or different sources cannot be used in combination.

For research use only, not for clinical diagnosis.

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