One-step Lentivirus Titer Kit, HIV-1 p24 Nimble90 ELISA

Catalog #: EA290002 (96 assays)

EA290002P5 (5x96 assays)

Principle of the Assay

The gag protein p24 (MW 24kD) is a major structural protein of the HIV capsid, which is required for virus particle assembly. There are approximately 2000 p24 molecules per virus particle, or at a molecule weight of 24 kDa, about 104 virus particles per picogram of p24. The onset of symptoms of AIDS correlates with an increased level of virus and p24 in the blood. HIV-1 p24 measurement is commonly used as an indicator of HIV-1 infection and viral load. In gene therapy, HIV-derived lentiviral vectors are commonly used to deliver transgenes to mammalian cells. Quantitative detection of the HIV-1 p24 has become a standard method for measuring lentiviral transduction efficiency.

This 90-minutes sandwich ELISA is used to measure HIV-1 p24 in cell culture or to determine the viral titer of lentiviral samples. Microtitration wells coated with anti-p24 capture antibody are exposed to p24 standard / test specimens and HRP conjugated p24 detection antibody mixture. The HIV-1 p24 antigen bound with HRP-conjugated detection antibody is specifically captured onto the immobilized antibody during incubation. After wash, specifically bound enzyme conjugate is detected by reaction with the Substrate Solution, tetramethylbenzidine (TMB). The assay is measured spectrophotometrically to indicate the level of HIV-1 p24 reactive determinants present in a sample.

Materials Supplied

The reagents supplied in this pack are for Research Use Only.

Description	Quantity
HIV-1 p24 Antibody Coated 96-well Strip Plate in foil pouch with desiccant	1
Recombinant HIV-1 p24 Standard (100ng/ml)	0.05 mL
p24 Detection Antibody-HRP Conjugate	2 mL
Lysis Buffer	2 mL
Assay Buffer	60mL
20x Plate Wash Buffer	30 mL
Substrate Solution (TMB)	12 mL
Stop Solution (1N HCl)	12 mL
Plate Sealer	1

Additional Materials Not Supplied

1. Horizontal orbital plate shaker capable of maintaining a speed of 450±50 rpm.
2. Microcentrifuge.
3. Multichannel pipette reservoir.
4. Disposable pipette tips to deliver volumes of 5µL to 200 µL (multichannel pipette preferred for dispensing reagents into microtiter plates).
5. Distilled or deionized water.
6. Disposable plastic/glass test tubes, approximate capacities 5mL and 15mL.
7. Absorbent paper towels.
8. Laboratory glassware consisting of 100 mL beaker, 100 mL and 1 L graduated cylinders, 5 mL and 10 mL pipettes.
9. Automatic microplate washer or laboratory wash bottle.
10. Microplate reader with 450nm filter.
11. Latex gloves, safety glasses and other appropriate protective garments.
12. Biohazard infectious waste containers.
13. Pipetting devices.
14. Timer.
15. 1% sodium hypochlorite as disinfectant. May be prepared from household bleach.

Related OriGene Products

1. Lenti-ORF, Plasmid and Ready-to-use Particles
2. Lenti-shRNA, Plasmid and Ready-to-use Particles
3. Lentiviral Packaging Kits, high efficiency
4. Lenti Concentrator, concentrate lentivirus in 2hrs

Storage and Stability

Upon receipt, store the kit at 2-8°C. The kit should not be used beyond the expiration date. Once opened, the unused microplate strips should be returned to their original foil pouch along with the desiccant. The diluted Wash Buffer should not be stored for longer than 3 weeks at 2-8°C. It is recommended that Wash buffer be freshly diluted before each assay. If the diluted Wash buffer becomes visibly cloudy during the 3 weeks, discard it. (Note: Concentrated Wash Buffer, when stored at 2-8°C, normally may develop crystalline precipitates, which can be re-dissolved at 37°C.)

The HIV-1 p24 Nimble90 ELISA kit may be considered to have deteriorated in any one of the following conditions:

1. Reagents are visibly cloudy.
2. The Substrate Solution turns blue, which is likely to be caused by chemical contamination.

Precautions

1. Keep in mind that the samples you are working with contain infectious virus. Follow the Centers for Disease Control & Prevention and the NIH guidelines to handle potentially infectious agents at the Bio safety Level 2.
2. Disposal or decontamination of fluid in the waste reservoir should be in accordance with guidelines described in the Department of Labor, Occupational Safety and Health Administration, occupational exposure to blood-borne pathogens; final rule (29 CFR 1910,1030) FEDERAL REGISTER, pp. 64176-84177,12/6/91.
3. The Substrate Solution and Stop Solution in this kit can irritate the skin and cause eye damage. Handle them with care and wear protective gloves, clothing and eye/face protection. Wash hands thoroughly after handling. Immediately flush the affected area with plenty of water in

case of contact with skin or eyes. Obtain medical attention if necessary.

Technical Hints and Suggestions

1. This kit should be used strictly according to the instructions in the Package Insert.
2. To ensure accurate results and avoid cross-contamination, use proper adhesive plate sealers during incubation steps, and change pipette tips when adding each standard and sample. Multi-channel pipettes are recommended for large assays.
3. To avoid reagents contamination, always use fresh pipette tips when drawing from stock reagent bottles.
4. Some reagents in the HIV-1 p24 ELISA kit are optimized for each kit lot. Do not exchange reagents from kits with different lot numbers.
5. Warm up the foil bag to room temperature before opening.
6. If plate shaker is not available, shake the plate by hand for 10 seconds to mix the solution in the well after adding the Lysis Buffer, Detection Antibody and Protein Standard /Samples, and increase the incubation time to 2 hours.
7. Without shaking during plate incubation period, the signal will be lower than expected, however, it has no significant influence on data analysis.
8. All reagents should be added to the plate in the same order.
9. If the Stop Solution does not mix thoroughly with the Substrate Solution, the color in the wells may appear green after adding stop solution. Gently tap the plate or pipette up and down to mix until the color in the wells change to yellow (avoid bubbles during this step).
10. Reagents should be dispensed with the tip of the micropipettes touching the side of the well at a point about mid-section. For automatic processors, follow manufacturer's recommendations.
11. It is recommended that all pipetting devices (manual or automatic), and thermometers are regularly calibrated according to the manufacturer's instructions.

Lentiviral Sample Collection and Storage

This assay is used to measure HIV-1 p24 in cell culture or to determine the viral titer of lentiviral samples.

Cell culture supernatants collection: Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Do not use self-defrosting freezers for sample storage. Frozen samples that have been thawed should be thoroughly mixed before testing.

Rinse Cycle

Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (300µL) using a squirt bottle, manifold dispenser, or automatic plate washer. Complete removal of liquid at each step is essential to good performance. After the last wash, invert the plate and blot it against clean paper towels.

Preparation for the Assay

1. *Standard Preparation*

Prepare standard 1 by diluting 10µL of standard stock (100ng/mL) into 490 µL (1:50 dilution) of Assay Buffer. This will give a final concentration of 2000 pg/mL as shown in Table 1.

Make 2x serial dilution of Standard 1 using Assay Buffer to generate a standard concentration range of 125 to 2000 pg/mL.

Table 1: p24 Standard Curve Preparation

Standard Number	Concentration of p24 (pg/mL)	P24 Standard (µL)	Assay Buffer (µL)
1	2000	10	490
2	1000	250 of #1	250
3	500	250 of #2	250
4	250	250 of #3	250
5	125	250 of #4	250
6	0		250

2. *Wash Buffer Preparation*

Dilute 20x Wash Buffer Concentrate to 1x with distilled or de-ionized water. It is recommended that only enough stock concentrate be diluted sufficient for immediate needs.

Assay Procedure

Note: All standards, controls and samples should be tested in duplicate.

1. Warm up kit to room temperature (18-25˚C).

2. Select sufficient microplate strips to accommodate all test samples, controls and reagent blank. Fit the strips into the holding frame.

3. Prepare 1:1 Lysis Buffer and Detection Antibody mixture: Add equal volume of Lysis Buffer and Detection Antibody to an Eppendorf tube or test tube, mix well. Calculate how much volume you need to prepare according to the reactions (wells) you are going to test (Total vol=1.1 x 20 x wells).

4. Add 20 µL of Lysis Buffer and Detection Antibody mixture into each well.

5. Add 100 µL of each standard, uninoculated (negative) and inoculated controls, and sample into appropriate wells. Depending on the titer of your lentivirus or specimen samples, dilution may be needed. The recommended dilution range for lenti-viral sample is from 1:200 to 500 in Assay Buffer. If the sample titer is not known, make serial dilution to titrate the sample.

6. Incubate for 1 hour at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

7. Wash the plate 5 times as described in the Rinse Cycle section.

8. Add 100 µL Substrate Solution into each well. A multichannel pipette should be used for best results. Incubate at room temperature (18-25˚C) and protect from light for 20 minutes.

9. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.

10. Read the absorbance values at 450 nm using a microplate reader blanked on the negative control well. If wavelength

correction is available, set second wavelength to 540 nm or 570 nm.

Calculation of Results

Average the duplicate readings for each standard and sample, and subtract the average zero standard optical density (O.D.).

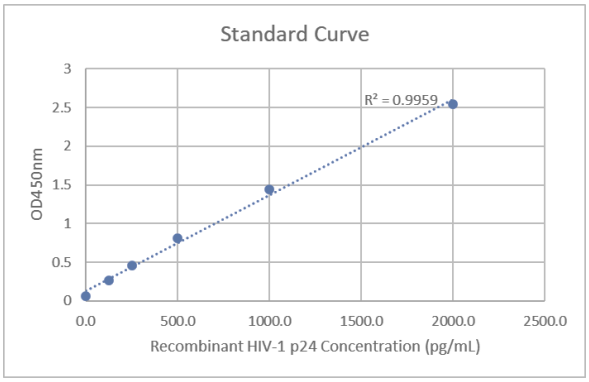
A 4-parameter logistic (4-PL) or a linear regression model providing a point-to-point curve fitting provides acceptable results. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) or a linear regression curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Do not force the line to be linear. The concentration of the samples can be found directly from the standard curve.

Table 2. Typical Data at 450nm.

Standards	450 nm absorbance
Standard 1 (2000 pg/mL)	2.5479
Standard 2 (1000 pg/mL)	1.4433
Standard 3 (500 pg/mL)	0.8113
Standard 4 (250 pg/mL)	0.4565
Standard 5 (125 pg/mL)	0.2646
Standard 6 (0 pg/mL)	0.0642

Typical HIV-1 p24 Nimble90 ELISA Kit Standard Curve

This standard curve was generated at OriGene for demonstration purpose only. A standard curve must be run with each assay.



Assay validation
The HIV-1 p24 assay should be considered valid if:
OD of the negative control $\leq$ 0.10
OD of the 1000 pg/ml standard $\geq$0.60

Performance Characteristics

1. Recovery
The recovery of HIV-1 P24 spiked to three different-levels of the assay range in diluted samples was evaluated.

Sample Type	Average % Recovery
Diluted lenti-viral sample	97%

2. Linearity
To assess the linearity of the assay, samples spiked with HIV-1 P24 were diluted with Assay Buffer to produce samples with values within the dynamic range of the assay.

Sample	% of Expected
1:2 spiked sample	95%
1:4 spiked sample	94%
1:8 spiked sample	92%

3. Sensitivity: 4.18pg/mL

4. Precision
Three samples with different levels of p24 were assayed 10 times each on three different assays. The intra-assay CV percentage and inter-assay CV percentage were calculated.

Intra-assay

Sample	%CV in Assay 1	%CV in Assay 2	%CV in Assay 3	Ave %CV
LV sample1 (n=10)	2.30	1.46	2.79	2.18
LV sample2 (n=10)	1.87	3.08	2.30	2.42
LV sample3 (n=10)	3.60	3.35	4.23	3.73

Inter-assay

Sample	Mean (pg/ml) in assay1	Mean (pg/ml) in assay2	Mean (pg/ml) in assay3	Ave (pg/ml)	SD	%CV
LV sample1 (n=10)	1564.80	1586.26	1606.39	1585.82	20.80	1.31
LV sample2 (n=10)	1209.85	1243.32	1220.37	1224.51	17.11	1.40
LV sample3 (n=10)	394.69	404.37	395.04	398.03	5.49	1.38

Determining Lentivirus Titer (TU)

Calculate the lentivirus titer using the values measured in the assay. The following calculations are based on approximately 2000 molecules of p24 per physical particle of lentivirus (LP).

- 1 LP contains: 2000 x 24 x 10^3Da/ (6 x 10^{23}) g= 8 x 10^{-5} pg of p24
- About 1 x 10^4 LP of lentivirus = 1 pg of p24.
- About 100 physical particles (LP) contain 1 transducing unit (TU). Therefore 1 pg/mL of p24 = 10^4 LP/mL= 100 TU/mL.

Multiply the results by the dilution factor to determine the actual HIV-1 p24 values in the samples.

Limitations of Use

1. **This kit is for research use only. Not for use in diagnostic procedures.**

2. The assay cannot be used to quantitate samples with p24 values higher than the highest standard without further dilution of the samples.

3. The p24 value measured using OriGene One-Step p24 Nimble90 ELISA kit may not be interchangeable with that obtained from other assay kits.

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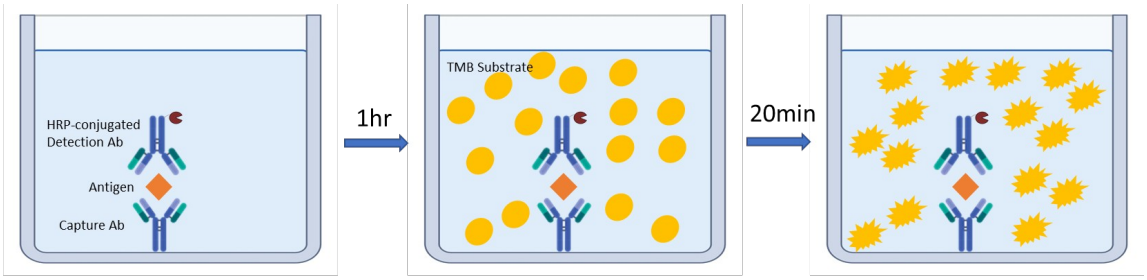
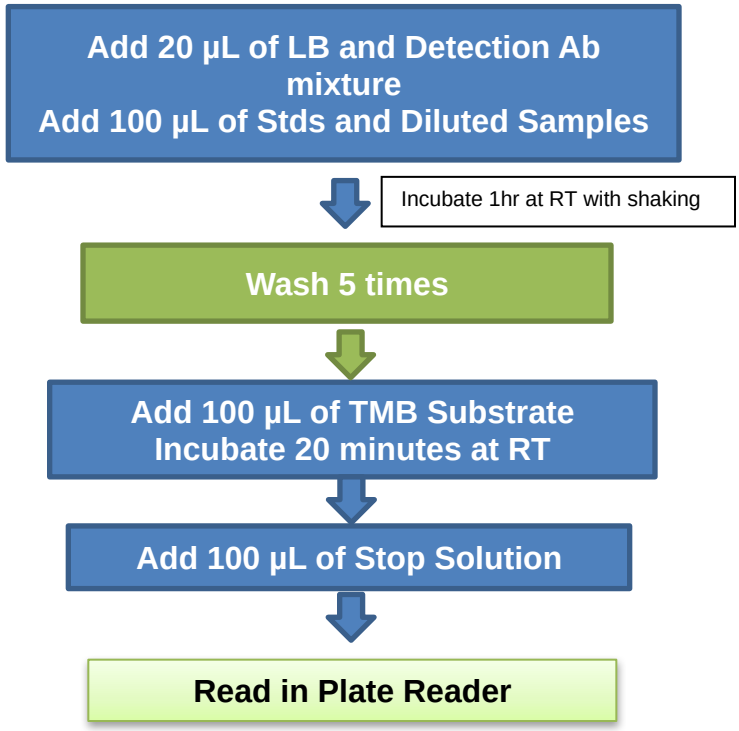
Version 022123

Nimble90 ELISA Assay Principle



Add sample and Detection Ab | Wash + add TMB substrate | Add stop solution and read

Assay Flowchart



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