

APPLICATION GUIDE

One-Wash™ Lentivirus Titer Kit, HIV-1 p24 ELISA

Catalog No.

TR30038 96 rxns (8x12 divisible strips)

TR30038P5 5 x 96 rxns

For quantitative detection of HIV-1 p24 Antigen in cell culture supernates for lentiviral particles titration

FOR RESEARCH USE ONLY
Not For Diagnostic Use

Assay Principle

OriGene's One-Wash™ Lentiviral Titer Kit, p24 ELISA can be used to measure the lentivirus titer; it is a standard sandwich enzyme-linked immunosorbent assay. First, the lentiviral particles in the tested samples are inactivated and the p24 antigen is released by the Lysis Buffer. A mouse monoclonal antibody specific for HIV-1 p24 is pre-coated onto 96-well plates. The p24 antigen in the specimen is specifically captured onto the immobilized antibody during specimen incubation. The captured antigen is then reacted with a biotinylated rabbit polyclonal HIV-1 p24 detection antibody. Subsequently, Streptavidin-HRP conjugate is added to produce visible color in the solution which is proportional to the amount of p24 captured from the specimen.

OriGene's One-Wash™ Lentiviral Titer Kit, ELISA is supplied for research purposes only. It is not intended for use in diagnostics.

Kit Components

Description	Quantity
HIV-1 p24 Antibody Coated 96-well Plate	1
Recombinant HIV-1 p24 Standard	100 µL
Biotinylated HIV-1 p24 Detection Antibody	10 mL
HRP Conjugated Streptavidin-Peroxidase	100 µL
Sample Lysis Buffer	5 mL
Assay Diluent	25 mL
10x Plate Wash Buffer	25 mL
TMB Substrate	10 mL
TMB Stop Solution	10 mL
Plate Sealer	2

Shipping and storage: shipped on blue ice, store at 2-8°C.

Materials Required but Not Supplied

1. Microplate reader in standard size.
2. Adjustable pipettes and disposable tips. Multichannel pipettes are recommended to handle the large number of samples.
3. Test tubes and racks for preparing samples and control dilutions.

4. Validated automatic microplate washer or manual vacuum aspiration equipment.
5. Timer.
6. 1% sodium hypochlorite as disinfectant. May be prepared from household bleach.
7. Distilled or deionized water.
8. Cell culture media

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

Precautions

1. Please follow biosafety level 2 guidelines when handling lentivirus samples.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Use universal precautions when handling kit components and test samples.
4. Duplicate well assay is recommended for both standard and sample testing.
5. HIV-1 p24 Antigen Standard contains sodium azide as a preservative. Sodium azide may react with metal pipes to form explosives. Flush pipes with large quantities of water upon disposal.
6. Stop solution contains hydrochloric acid which may cause severe burns. In case of contact with eyes or skin, rinse immediately with water and seek medical assistance.

Preparation of Reagents

1. HIV-1 p24 Antigen Standard: Prepare a series of six standards from the HIV-1 p24 Antigen Standard. Use the dilution scheme in the following table. Please discard the diluted standard.

Standard Number	Concentration of p24 (pg/ml)	HIV-1 p24 Antigen Standard (µl)	Assay Diluent (µl)
1	400	50	950
2	200	500 of #1	500
3	100	500 of #2	500
4	50	500 of #3	500
5	25	500 of #4	500
6	0	0	500

- Streptavidin-Peroxidase: To prepare the Streptavidin-Peroxidase Working Solution, use the dilution schemes in the following table. Any diluted Streptavidin-Peroxidase Working Solution remaining after the completion of the assay must be discarded.

Number of Strips used	SA-HRP (µl)	Assay Diluent (ml)
3	20	4
6	35	7
9	50	10
12	60	12

- Plate Wash Buffer: Dilute 10X Plate Wash Buffer 1:10 in distilled or deionized water prior to use. 1X Plate Wash Buffer may be stored at 2° - 8°C for up to 1 week.

- Sample preparation

The samples are diluted (1:1500 or 1:2000) in cell culture medium.

Treat samples in a test tube by pipetting 50ul lysis buffer into 450ul sample and mix well.

Test Procedure

Allow all reagents to reach room temperature before use. Label test tubes to be used for the preparation of standards and samples. Label each strip on its end tab to identify the strips should they become detached from the plate frame during the assay. Place surplus strips and desiccant into the Resealable Plastic Bag, seal and store at 2-8°C.

- Leave one well of the microtiter plate empty during the assay. This well is used for a substrate blank.

2. Leave one well of the microtiter plate filled with cell culture medium. This well is used for medium blank.
3. Pipet 200 µl of standards #1-6 into duplicate wells.
4. Pipet 200 µl of each sample into duplicate wells.
5. Cover microplate with a plate sealer and incubate 2 hrs to overnight at room temperature (no difference comparing at 37°C).
6. Discard the liquid in the plate, and blot the plate onto paper towels or other absorbent material. DO NOT let the wells completely dry at any time.
7. Pipet 100 µl of the HIV-1 p24 Detection Ab into each well, except the substrate blank. Cover the microplate with a sealer and incubate for 1 hour at room temperature.
8. Discard the liquid in the plate, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
9. Pipet 100 µL of the Streptavidin-Peroxidase Working Solution into each well except the substrate blank. Cover the microplate with a sealer and incubate for 30 minutes at room temperature.
10. Discard the liquid in the plate, and blot the plate onto paper towels or other absorbent material.
11. Wash each well 6 times with 300 µL of 1X Plate Wash Buffer and discard. Thoroughly blot by striking inverted microplate or strips on a pad of absorbent towels. Continue striking until no droplets remain in the wells.
12. Pipet 100 µL of Substrate into all wells and incubate in dark for 30 minutes at room temperature (18°- 25°C). A blue color will develop in wells containing viral antigen.
13. Stop the reaction by pipetting 100 µL of Stop Solution into each well. A color change from blue to yellow will result.
14. Within 15 minutes, read the optical density of each well at 450 nm using a microplate reader.

Test Validity

Determine the mean optical density values for each standard and specimen. For the test to be valid, it must meet the following criteria:

1. The mean optical density of the 0 pg/mL standard and the substrate blank must be less than 0.100.
2. The mean optical density of the 200 pg/ml standard must be greater than or equal to 0.500.

Calculation and Interpretation of Results

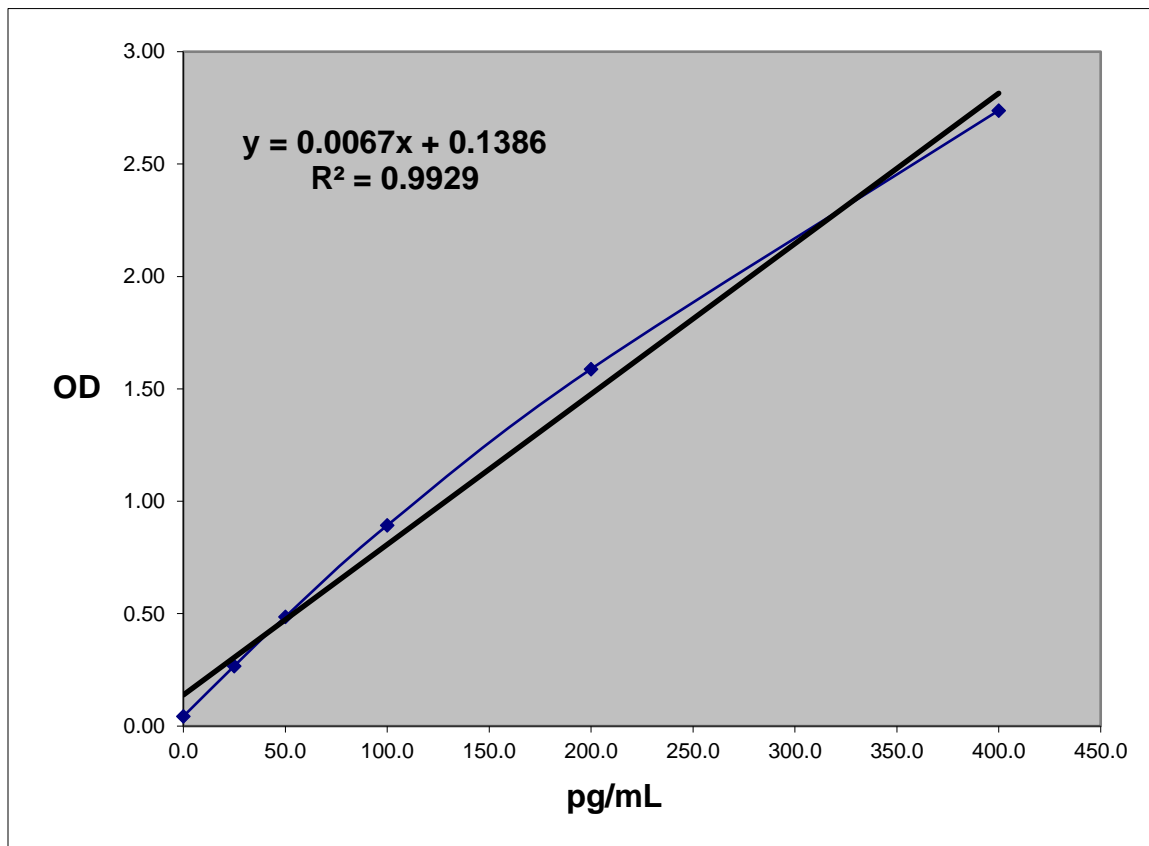
1. Typical Data Obtained from HIV-1 p24 Antigen Standard

Concentration (pg/mL)	0	25	50	100	200	400
OD	0.04	0.27	0.49	0.89	1.59	2.74

2-hour assay OD values may be less than the overnight results. Standard curves may vary as a result of incubation time and temperature, laboratory temperature, etc.

2. Typical HIV-1 p24 Antigen ELISA Kit Standard Curve.

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



3. Lentivirus titer (transducing units per milliliter (TU/mL)) calculation:

- Average the duplicate readings for each standard, and sample.

- For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). Then determine the concentration of HIV-1 p24 antigen in the samples by interpolation from the standard curve. Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.
- Use formula $10^7 \text{ TU/mL} = 1 \times 10^5 \text{ pg/mL}$ to calculate lentivirus particle titer in each tested sample. There are approximately 2000 molecules of p24 per physical particle (PP) of lentivirus.
 - 1 PP contains $8 \times 10^{-5} \text{ pg}$ of p24 (derived from $2000 \times 24 \times 10^3 \text{ Da} / (6 \times 10^{23} \text{ g})$).
 - 1×10^4 PP of lentivirus for every pg of p24 antigen.
 - About 100 physical particles (PP) contain 1 transducing unit (TU). Therefore $10^7 \text{ TU/mL} = 10^9 \text{ PP/mL} = 1 \times 10^5 \text{ pg/mL}$.

Recommended Plate Layout

Before starting, it is recommended that a plate plan be designed. Such a plan will assist in assay workflow and data analysis. A suggested plate plan is shown below.

	1	2	3	5	6	7	8	9	10	11	12
A	Substrate	Substrate									
B	Medium	Medium									
C	Std 1	Std 1									
D	Std 2	Std 2									
E	Std 3	Std 3									
F	Std 4	Std 4									
G	Std 5	Std 5									
H	Neg	Neg									

Sample Wells

Running all standards, samples, and controls in duplicate or triplicate is recommended.

Procedure Outline (Option2)

