

Version 01

Update: 08/04/2023

Product Manual

Lentivirus Titer p24 ELISA Kit Pro

Cat. No. L00974

For Research Use Only. Not for Use in Diagnostic Procedures.

The operator should read technical manual carefully before using this product.

Contents

I. DESCRIPTION	2
II. ASSAY PRINCIPLE.....	2
III. KIT CONTENTS	3
IV. STORAGE.....	4
V. REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED	4
VI. PRECAUTIONS	4
VII. SPECIMEN COLLECTION AND STORAGE	5
VIII. PROTOCOL.....	5
● Reagent Preparation	5
● Sample preparation.....	6
● Capture Plate Preparation	7
● Test Procedure.....	8
IX. ASSAY PROCEDURE SUMMARY	10
X. INTERPRETATION OF RESULTS.....	11
● Assay Validation	11
● Suggested Calculation of Data	11
● Lentivirus Titer Calculation	12
XI. Analytical Performance	13
● Linear Range	13
● Detection Capability.....	13
● Measurement Precision	13
● Recovery	14
XII. Example of Results for Detecting Lentivirus-Associated p24.....	14
● Removal Capability of L00974 for Free p24	14
● Specificity of L00974 in Detecting Lentivirus-Associated p24	15
● Accuracy of L00974 in Detecting Lentivirus Samples during Production	15
XIII. TROUBLESHOOTING.....	16
XIV. REFERENCES.....	18

I. DESCRIPTION

Lentivirus vector is derived from human immunodeficiency virus-1 (HIV-1), which has been widely used to transfer gene(s) of interest into dividing and non-dividing cells. The lentiviral titer, as assessed by the p24 protein content, is an essential characterization procedure for routine quality control and lot release, as well as for purification process optimization. However, HEK-293T cells will release free p24 protein into the supernatant during transfection, and this part of p24 would account for a large percentage of the total p24. The traditional p24 ELISA kit detects both lentivirus associated p24 and free p24, which will overestimate the titer of the lentivirus.

GenScript's Lentivirus Titer p24 ELISA Kit Pro (Cat. No. L00974) is a Sandwich ELISA designed for quantitatively measuring the physical titer of any HIV-1 based lentivirus in cell culture supernatant. After removing free p24 (patented technology), the amount of lentivirus-associated p24 can be measured by the p24 ELISA kit. The kit has the same linear range, detection capability, and precision with Lentivirus Titer p24 ELISA Kit (Cat. No. L00938) in the detection of p24 concentration. This kit makes lentivirus titration easy, accurate and reproducible.

II. ASSAY PRINCIPLE

The Lentivirus Titer p24 ELISA Kit Pro consists of two parts, including *Lentivirus Free p24 Removal Reagent* (patented technology) and Lentivirus Titer p24 ELISA Kit. Before detecting the concentration of p24 protein, free p24 in lentiviral supernatant can be specifically removed by *Lentivirus Free p24 Removal Reagent*.

The Lentivirus Titer p24 ELISA Kit utilizes two HIV-1 p24 monoclonal antibodies which bind to different epitopes of the p24 protein. When standards or samples are added to the capture plate, the p24 protein in the sample can be captured on the plate coated with the HIV-1 p24 capture antibody. Then the HIV-1 p24 detection antibody conjugated with biotin is added to interact with the p24 protein bound on the plate. Streptavidin-Horseradish Peroxidase conjugate (Streptavidin-HRP) is added to interact with the biotin-conjugated anti-p24 antibody (Biotin Anti-p24 Antibody). After washing steps, 3,3',5,5'-Tetramethylbenzidine solution (TMB Solution) is added resulting in the formation of blue color. The reaction is stopped by adding Stop Solution. Application of the Stop Solution results in the color changing from blue to yellow. The intensity of the color can be read at 450 nm by a microplate reader.

The quantity of p24 protein in the sample is precisely quantified against a p24 standard curve. p24 values are then correlated to the lentivirus titer of packaging cell culture supernatants.

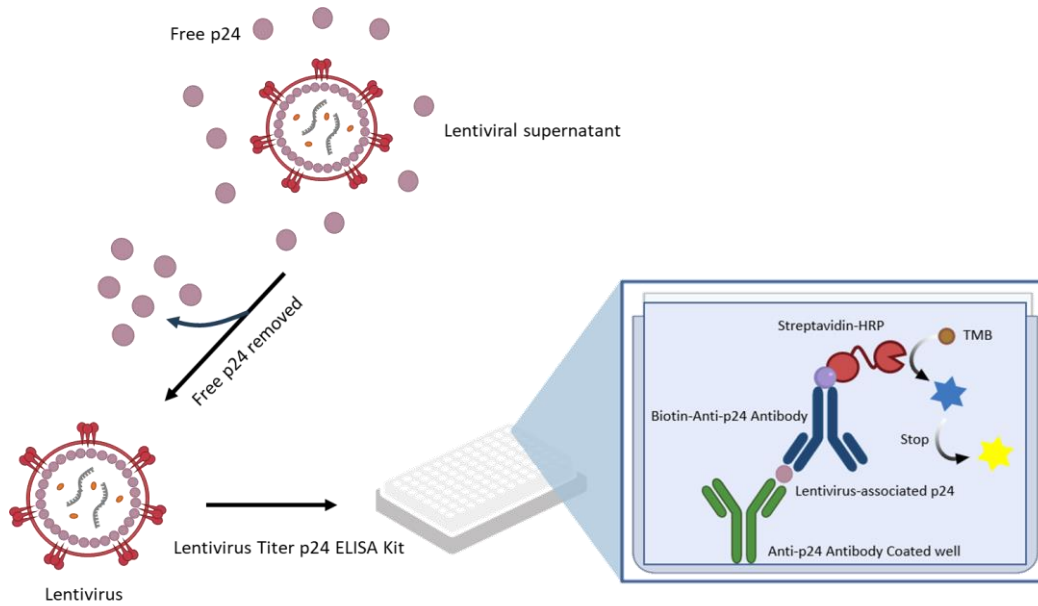


Figure 1: Schematic diagram of Lentivirus Titer p24 ELISA kit Pro

III. KIT CONTENTS

The kit provides the following reagents and solutions required for lentivirus titration in samples.

Component	Quantity/Size	Part No.
Capture Plate	1 plate	E1-80
Standard Stock	1 vial (100 μ L)	E1-10
Lysis Solution	1 vial (1.6 mL)	E1-A0
Biotin Anti-p24 Antibody	1 bottle (12 mL)	E1-20
Streptavidin-HRP	1 bottle (12 mL)	E1-30
Lentivirus Free p24 Removal Reagent	1 bottle (2 mL)	E1-40
10 \times Beads Wash Solution	1 bottle (40 mL)	E1-50
TMB Solution	1 bottle (12 mL)	A1-40
20 \times Wash Solution	1 bottle (40 mL)	A1-70
Stop Solution	1 bottle (6 mL)	A1-50
Plate Sealer	2 pieces	N/A

- Capture Plate: 96 well microplates (8 wells x 12 strips); 12 strips are configured in plate; plate is sealed in a foil pouch with a desiccant.
- Standard Stock contains 2 μ g/mL of recombinant p24 protein. The Standard Stock shall be diluted to generate p24 standard curve.

IV. STORAGE

The unopened kit is stable for at least 12 months from the date of manufacture at 2°C to 8°C, and the opened kit is stable for up to 1 month from the date of opening at 2°C to 8°C.

V. REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

- Cell culture medium (For example, DMEM culture media with 10% Fetal Bovine Serum (FBS))

This reagent is used as a dilution buffer for both samples and standards. If the user does not have this reagent, the **1× Wash Solution** can be used as an alternative dilution buffer for samples and standards after the removal of the free p24. The preparation instruction for the **1× Wash Solution** is detailed in the PROTOCOL-Reagent Preparation-1× Wash Solution.
- Microplate reader capable of measuring absorbance at 450 nm
- Automated microplate washer
- Deionized or distilled water
- Graduated cylinder
- Plastic container
- Tubes to aliquot and dilute samples
- 10 µL, 200 µL, and 1000 µL precision pipettes
- 10 µL, 200 µL, and 1000 µL pipette tips
- Multichannel pipettes
- Disposable reagent reservoir
- Absorbent paper
- Laboratory timer
- Refrigerator
- Centrifuge
- 25 ± 2°C incubator
- Magnetic separation rack
- Mixing/Rotation device

VI. PRECAUTIONS

1. The VSV-G-protein pseudotyped lentiviral vectors (VSV-G-LVs) from HIV-1-based vectors are capable of infecting human cells. The viral supernatants produced by these lentiviral systems contain potentially hazardous recombinant viruses. Follow the recommended NIH guidelines

- for all materials containing BSL-2 organisms.
2. Any materials that may be contaminated with potentially infectious materials should be treated as infectious materials in accordance with local regulations; For more detailed information, please contact your local lab safety committee.
 3. All personnel working with lentivirus must complete appropriate Biosafety Training.
 4. Reagents that contain preservatives may be toxic if ingested, inhaled, or spilled on skin.
 5. Avoid contact of skin, eyes, or clothing with Stop Solution or TMB Substrate. Keep the container tightly closed. In case of an accident, please seek medical advice immediately.
 6. Do not use the kit if there is any visible damage to the packaging or kit contents.
 7. Do not mix components from different batches. Do not mix with components from other manufacturers.
 8. Do not use reagents beyond the stated expiry date.
 9. All reagents must be equilibrated to room temperature (20° - 25°C) before running the assay. Only take an appropriate amount of reagents at once. Do not put unused reagents back into the vials as reagent contaminations may occur.
 10. The temperature for the reaction should be maintained at 25°C.
 11. Before opening the Standard Stock, quickly span the vial to ensure that all the liquid has collected at the bottom, and prevent the liquid from splashing when opening the lid.
 12. Use only distilled or deionized water and clean glassware.
 13. Do not let wells dry during the test; add reagents immediately after completing the washing steps.
 14. Do not let the beads in Lentivirus Free p24 Removal Reagent dry before use.
 15. Dilution of the samples for ELISA detection should be done after the removal of free p24.

VII. SPECIMEN COLLECTION AND STORAGE

1. The handling and storage information provided here is intended to be used as a general guideline. Sample stability has not been evaluated. When samples need to be stored for a long time, users need to evaluate the stability of the samples. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria that meet their needs.
2. Store specimens at -20°C or lower if not tested immediately. Avoid repeated freeze-thaw cycles.

VIII. PROTOCOL

- **Reagent Preparation**

All reagents must be equilibrated to room temperature before use (20°C to 25°C). All samples

and reagents should be vortexed before use. Store all reagents back in the refrigerator promptly after use.

1× Beads Wash Solution: Dilute the 10× Beads Wash Solution with deionized or distilled water with a volume ratio of 1: 9. For example, dilute 40 mL of 10× Beads Wash Solution with 360 mL of deionized or distilled water to make 400 mL of 1× Beads Wash Solution. Store the solution at 2°C to 8°C when not in use.

1× Wash Solution: Dilute the 20× Wash Solution with deionized or distilled water with a volume ratio of 1:19. For example, dilute 40 mL of 20× Wash Solution with 760 mL of deionized or distilled water to make 800 mL of 1× Wash Solution. Store the solution at 2°C to 8°C when not in use.

Note: If any precipitate is found in the 10× Beads Wash Solution or 20× Wash Solution, incubate the bottle in a water bath (up to 50°C) with occasional mixing until all the precipitate is dissolved.

- **Sample Preparation**

- Removal of Free p24**

1. Suspend the beads in Lentivirus Free p24 Removal Reagent thoroughly and transfer 20 µL Lentivirus Free p24 Removal Reagent into an empty tube.

Note: The beads in Lentivirus Free p24 Removal Reagent settle quickly, so it need to be transferred immediately after resuspension.

2. Place the tube into a magnetic separation rack for 5-10 seconds to collect the beads and discard the supernatant from the tube with a pipette.
3. Add 1 mL 1× Beads Wash Solution to the tube and invert the tube several times to wash the beads.
4. Place the tube into the magnetic separation rack for 5-10 seconds and remove the supernatant from the tube with a pipette.
5. Repeat the steps 3 to 4 for another two times, and make sure that the beads are washed completely.
6. Add 500 µL lentiviral cell culture supernatant to the prepared magnetic beads.

*Note: Normally, free p24 in lentiviral supernatant can be completely removed **without dilution**. However, if dilution is necessary, it is recommended to use a **complete medium**. Diluting the virus with deionized water or PBS may reduce the efficiency of removal. **Don't Dilute the virus with 1×washing solution or other solutions capable of lysing the lentivirus.***

7. Mix by rotation on a tube rotator for 15 min at room temperature.
8. Place the tube into the magnetic separation rack for 5-10 seconds to collect the free p24 removed lentiviral supernatant.

- Sample Dilution**

Use fresh cell culture medium to dilute the lentiviral supernatant.

Unknown samples, especially those samples with very high titer of p24 (i.e. >2000 pg/mL or 2.50E+07 LP/mL) must be diluted before the assay to obtain an accurate titer value of p24 which is within the linear range of the kit. We recommend several serial 10-fold dilutions for unknown samples to ensure that at least one diluted sample is in the range of the standard curve. Usually, 10-10,000 fold dilutions of the sample are required.

It is recommended that all samples be prepared in duplicate. The results are multiplied by the dilution factor to determine the p24 value in the original sample, as described in **X. INTERPRETATION OF RESULTS, Lentivirus Titer Calculation.**

Standard Working Solution

Centrifuge Standard Stock at 10,000 rpm for several seconds.

Dilute Standard Stock with fresh cell culture medium with a volume ratio of 1:1000. The diluent used to dilute the standard must be the same as the samples. For the dilution of standard, mix 5 μ L of Standard Stock with 500 μ L of fresh cell culture medium first, and then dilute 100 μ L of the mixture with 900 μ L of fresh cell culture medium to make 1 mL of 2000 pg/mL Standard working solution with a label of "Std-01". The recommended dilution gradient is as follows: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/mL, which is used to establish a standard curve.

Prepare seven 1.5 mL tubes labeled numerically from Std-02 to Std-08 consecutively, and pipette 500 μ L of fresh cell culture medium into each tube.

Pipette 500 μ L of 2000 pg/mL standard solution from Std-01 tube into the Std-02 tube and mix to make 1000 pg/mL of Standard working solution. Then pipette 500 μ L of the solution from the former tube into the latter one according to these steps.

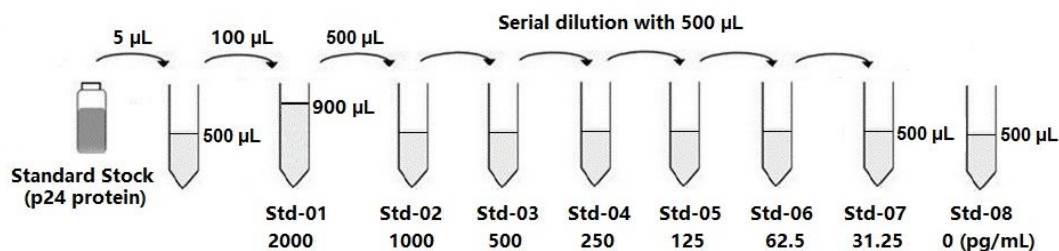


Figure 2: Diagram of Serial Dilution.

● Capture Plate Preparation

1. It is recommended that all standards be prepared at least in duplicate.
2. Count the strips according to the number of test samples and install the strips. Make sure the strips are tightly snapped into the plate frame.
3. Leave the unused strips in the foil pouch and store at 2°C to 8°C. The strips must be stored in the closed foil pouch to prevent moisture from damaging the Capture Plate.

Table 1. Setup of p24 standards and samples on the Capture Plate

	Standards		Samples									
	1	2	3	4	5	6	7	8	9	10	11	12
A	Std-1	Std-1	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	Std-2	Std-2	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	Std-3	Std-3	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	Std-4	Std-4	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	Std-5	Std-5	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	Std-6	Std-6	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	Std-7	Std-7	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	Std-8	Std-8	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Std: Standard number; **S:** Sample number

- **Test Procedure**

The incubation time of this kit is reduced to 1 hour 40 minutes, requiring only 2.5 hours to complete the titration process.

Standards and Samples Incubation

1. Add 10 μ L of Lysis Solution to all the wells in the Capture Plate.
2. Add 100 μ L of standard working solutions and samples to the corresponding wells in the Capture Plate. Mix by placing the plate on a rotary shaker for 30 - 60 seconds.
3. Cover the plate with Plate Sealer and incubate at 25°C for 60 minutes.
4. Remove the Plate Sealer and wash the plate with 260 μ L of 1 \times Wash Solution four times.
5. Tap the inverted plate onto absorbent paper to remove residual liquid in the wells after the wash steps.

Detection Antibody Incubation

6. Add 100 μ L of Biotin Anti-p24 Antibody to all the testing wells.
7. Cover the plate with Plate Sealer and incubate at 25°C for 15 minutes.
8. Remove the Plate Sealer and wash the plate with 260 μ L of 1 \times Wash Solution four times.
9. Tap the inverted plate onto absorbent paper to remove residual liquid in the wells after the washing steps.

Enzyme Conjugate Incubation

10. Add 100 μL of Streptavidin-HRP to all the testing wells.
11. Cover the Plate with Plate Sealer and incubate at 25°C for 10 minutes.
12. Remove the Plate Sealer and wash the plate with 260 μL of 1 \times Wash Solution four times.
13. Tap the inverted plate onto absorbent paper to remove residual liquid in the wells after the washing steps.

Substrate Reaction and Absorbance Measurement

14. Add 100 μL of TMB Solution to each well and incubate the plate in the dark at 25°C for 15 minutes (start timing after the addition of TMB Solution to the first well).
15. Add 50 μL of Stop Solution to each well to stop the reaction.
16. Read the absorbance in the microplate reader at 450 nm immediately.

IX. ASSAY PROCEDURE SUMMARY

- 1 • Aspirate 20 μ L Lentivirus Free p24 Removal Reagent, and wash the beads in the reagent for three times
- 2 • Add the lentivirus cell culture supernatant to the prepared beads for 15 min.
- 2 • Collect the free p24 removed lentiviral supernatant, and dilute the lentiviral supernatant samples or a set of standards with cell culture medium.
- 3 • Add 10 μ L of Lysis Solution and 100 μ L of the diluted samples/standards to the corresponding wells and mix on rotary shaker for 30 - 60 seconds. Incubate the plate at 25°C for 60 minutes.
- 4 • Wash the plate with 260 μ L of 1 \times Wash Solution per well four times.
- 5 • Add 100 μ L of the Biotin Anti-p24 Antibody to the well and incubate at 25°C for 15 minutes.
- 6 • Wash the plate with 260 μ L of 1 \times Wash Solution per well four times.
- 7 • Add 100 μ L of the Streptavidin-HRP and incubate at 25°C for 10 minutes.
- 8 • Wash the plate with 260 μ L of 1 \times Wash Solution per well four times.
- 9 • Add 100 μ L of TMB Solution and incubate the plate in dark at 25°C for 15 minutes.
- 10 • Add 50 μ L of Stop Solution to each well to stop the reaction.
- 11 • Read the plate immediately.

X. INTERPRETATION OF RESULTS

- **Assay Validation**

To ensure the validity of results, the following criteria noted in Table 2 are required. If a test fails to meet the requirements, the test is invalid and must be repeated.

Table 2. OD450 values for quality control

Items	Special Test	Requirements
1	OD450 value for 2000 pg/mL standard	> 1.5
2	OD450 value for 0 pg/mL standard	< 0.1

Note: The standards in the table are only intended to evaluate the performance of the kit.

- **Suggested Calculation of Data**

Statistical software can be used to generate the standard curves. Choose a method with a high goodness of fit (R^2) to analyze the data, such as a four-parameter logistic (4-PL) model that provides point-to-point curve fitting.

Manual method for demonstration:

The standard curve has been provided for demonstration only. It should be prepared each time an assay is performed.

1. Plot the standard curve with the p24 protein concentration (pg/ml) on the x-axis and the corresponding mean absorbance value on the y-axis.
2. Using a four-parameter logistic curve fitting program, calculate the best-fitting linear line through the points of the standard curve.

The equation for the model is:

$$y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$$

A = the minimum value that can be obtained (i.e. what happens at 0 dose)

D = the maximum value that can be obtained (i.e. what happens at infinite dose)

C = the point of inflection (i.e. the point on the S shaped curve halfway between a and d)

B = Hill's slope of the curve (i.e. this is related to the steepness of the curve at point c)

3. Determine the p24 concentration (pg/mL) for the samples with the standard curve.

Table 3. Sample data for the standard curve.

Standard		Absorbance (450 nm)		
(pg/mL)	(LP/mL)	Duplicate 1	Duplicate 2	Average
2000	2.50E+07	2.0795	2.0740	2.0768
1000	1.25E+07	1.0606	1.1114	1.0860
500	6.25E+06	0.5789	0.5988	0.5889
250	3.13E+06	0.3097	0.3186	0.3142
125	1.56E+06	0.1644	0.1784	0.1714
62.5	7.81E+05	0.1081	0.1168	0.1125
31.25	3.91E+05	0.0741	0.0737	0.0739
0	0	0.0445	0.0435	0.0440

Standard curve as a demonstration

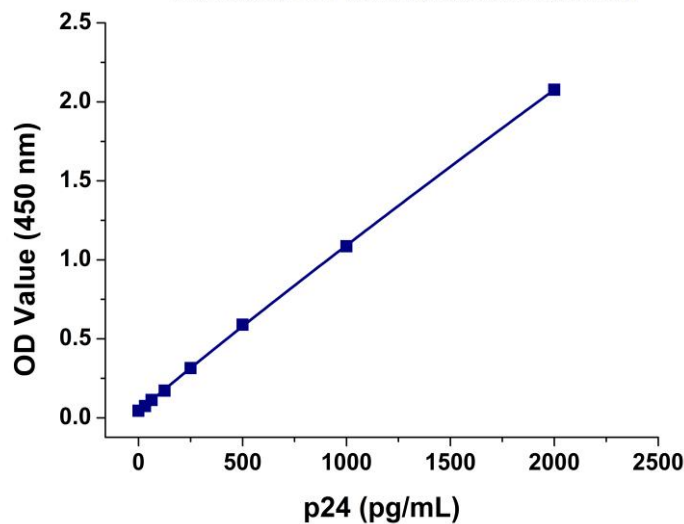


Figure 3: Lentivirus Titer p24 ELISA kit standard curve.

- **Lentivirus Titer Calculation**

1. Determine lentivirus associated p24 amount in the initial lentivirus sample with the formula below.

$$\text{p24 Concentration (Virus associated p24, pg/mL)} = \text{p24 (pg/mL)} \times \text{Dilution Factor}$$

2. Calculate lentivirus titer (physical titer) with the below formula.

$$8 \text{ to } 80 \text{ ng/mL} = 10^{8-9} \text{ LP/mL} = 10^6 \text{ TU/mL}$$

Note: The formula is based on that each lentiviral particle (LP) contains approximately 2,000 molecules of p24. 1 LP contains $2000 \times 24 \times 10^3 / (6 \times 10^{23})$ g of p24 = 8×10^{-5} pg of p24 or 1 ng p24 =

1.25×10^7 LPs. For reasonably packaged lentivirus vectors, 1 TU is about 100 to 1000 LP [3-5]. The result is the physical titer of the lentivirus calculated from the p24 protein level. When determining infectious titers, the results are related to the target cell line type or transduction method.

XI. Analytical Performance

- **Linear Range**

The linear range of this kit is from 31.25 to 2000 pg/mL according to the evaluation method recommended in CLSI guideline EP6-A [1].

- **Detection Capability**

According to the evaluation method recommended in CLSI guideline EP17-A2[2], the LOB (Limit of Blank) of this kit is 14.13 pg/mL, the LoD (Limit of Detection) is 29.42 pg/mL and the LoQ (Limit of Quantitation) is 39.03 pg/mL.

- **Measurement Precision**

Intra- and inter-assay precision were measured in 3 pools of different concentrations, using 3 lots of kits. From the assay, the intra-assay % CVs are less than 10%, and the Inter-assay % CVs are less than 15%, indicating the kit has good intra- and inter-assay precision.

Table 4: Intra-assay CV evaluation with three-batch Lentivirus Titer p24 ELISA Kit

Batch No.	Repeats	Average Conc. pg/mL	%CV
#1	10	215.55	3%
#1	10	829.45	4%
#1	10	1537.42	4%
#2	10	209.60	5%
#2	10	792.76	2%
#2	10	1431.42	3%
#3	10	188.09	6%
#3	10	759.99	4%
#3	10	1377.71	3%

Table 5: Inter-assay CV evaluation with three-batch Lentivirus Titer p24 ELISA Kit

Batch Amount	Repeats	Average Conc. pg/mL	%CV
3	3×10	204.41	7%
3	3×10	794.07	5%
3	3×10	1448.85	6%

- **Recovery**

Recovery experiments mean adding a known amount of p24 to the buffer matrix and then testing whether the added p24 can be recovered quantitatively. Normally, an 80% to 120% recovery rate can be regarded as there being relatively slight interference from the diluent or matrix. Extreme pH, high salt concentrations, some high protein concentrations, and some detergents may result in inadequate recovery. The user can dilute the standards provided in this kit into the sample matrix of interest and perform recovery experiments to identify whether the sample can be accurately recovered in the matrix. This is necessary to determine the accuracy of the experiment.

From the assay in triplicates, the recovery rates of GenScript Lentivirus Titer p24 ELISA kit are all located in the range of 80% to 120%, which indicates lentivirus titration with this ELISA kit is accurate.

Table 6: Recovery rate of Lentivirus Titer p24 ELISA kit in triplicates

Batch No.	Repeats	Average Conc. pg/mL	Recovery Rate
#1	3	1945.30	86%
#2	3	1787.64	108%
#3	3	1752.76	90%

XII. Example of Results for Detecting Lentivirus-Associated p24

- **Removal Capability of L00974 for Free p24**

Different concentrations of p24 protein were added to the DMEM culture media with 10% FBS and were detected using a traditional p24 ELISA kit or Lentivirus Titer p24 ELISA Kit Pro which contains free p24 removal process. The results showed that after the removal of p24 protein by Free p24 Removal Reagent in L00974, the p24 protein was substantially undetectable in the supernatant, indicating that Lentivirus Free p24 Removal Reagent can effectively remove free p24.

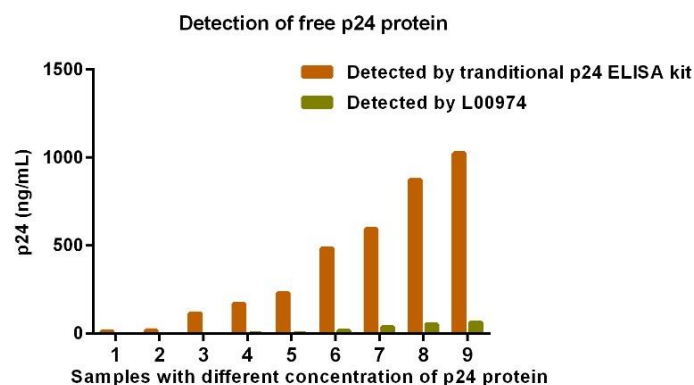


Figure 4 Different concentrations of p24 protein in cell culture media were detected by traditional p24 ELISA kit or Lentivirus Titer p24 ELISA Kit Pro

- **Specificity of L00974 in Detecting Lentivirus-Associated p24**

Various concentrations of free p24 protein were introduced into purified lentivirus supernatant, and the samples were then analyzed using traditional p24 ELISA Kit or Lentivirus Titer p24 ELISA Kit Pro, which includes a step to eliminate free p24 protein. The results showed that the free p24 was effectively removed from the mixture of free p24 protein and purified lentivirus supernatant and the concentration of p24 detected by Lentivirus Titer p24 ELISA Kit Pro was consistent with actual lentivirus-associated p24.

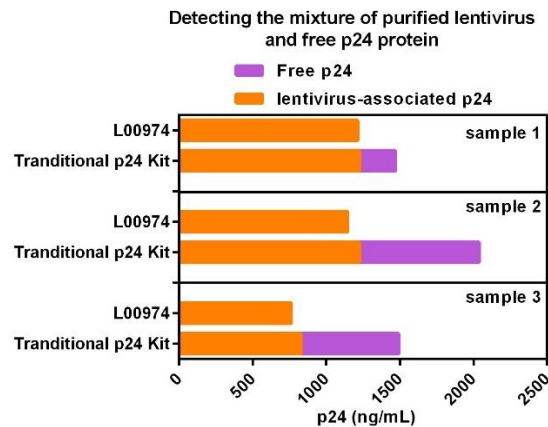


Figure 5 Samples containing different ratios of free p24 and purified lentivirus were detected by traditional p24 ELISA kit or Lentivirus Titer p24 ELISA Kit Pro

- **Accuracy of L00974 in Detecting Lentivirus Samples during Production**

Different lentivirus samples at the same production stage were analyzed using either the traditional p24 ELISA Kit or the Lentivirus Titer p24 ELISA Kit Pro. The results revealed variations in the proportion of free p24 among different lentivirus samples, with some exceeding 20%. Additionally, the concentration of p24 detected by the Lentivirus Titer p24 ELISA Kit Pro was found to be consistent with the actual lentivirus-associated p24 levels, which will be a benefit for quality control and lot release of lentivirus.

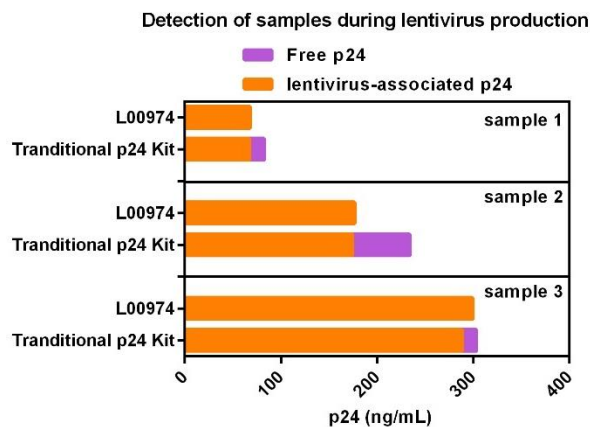


Figure 6 Different lentivirus samples at the same production stage were analyzed by traditional p24 ELISA kit or Lentivirus Titer p24 ELISA Kit Pro

XIII. TROUBLESHOOTING

Problem	Probable Cause	Solution
Poor Precision	Wells are not washed or aspirated properly	Make sure the washing apparatus works properly and wells are dry after aspiration
	Wells are scratched with pipette tip or washing needles	Dispense and aspirate solution into and out of wells with caution
	Particulates are found in the samples	Remove any particulates by centrifugation prior to the assay
Weak/No Signal	Substrate is not added or added at the wrong time	Follow the manual to add the substrate properly
	Components are used from other lots or sources	Use only lot-specific components
	Substrate is contaminated	Use a new Substrate from the same Lot
	Volumes of reagents are not correct	Repeat the assay with the required volumes as noted in the manual
	The plate is not incubated for proper time or temperature	Follow the manual to repeat the assay
	The plate is not read within the specified time range	Read the plate within 5 minutes
High Background	Plate is not washed properly	Make sure the washing apparatus works properly
	Substrate is contaminated	Use new substrate from the same Lot
	Evaporation of wells during incubations	Perform incubation steps with a plate sealer in a repeat assay
	Incorrect incubation times and/or temperatures	Follow the manual to repeat the assay
Free p24 is removed inefficient or excessive	When removing free p24, the lentiviral supernatant is diluted with PBS, deionized water, 1×washing solution or solutions capable of lysing the lentivirus	Use the lentivirus culture supernatant directly or dilute the lentivirus with a complete culture medium
	Incorrect incubation times and/or temperatures during free p24	Follow the manual to repeat the assay

	removal	
	Magnetic beads are aspirated Excessive or insufficient	Transfer the magnetic beads immediately after resuspension.

XIV. REFERENCES

1. CLSI document EP06-A (Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline, 2nd Edition).
2. CLSI document EP17-A2 (Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition).
3. Kahl CA, 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 Forest virus[J]. Journal of virology, 2004, 78(3): 1421-1430.
4. White S M, Renda M, Nam N Y, et al. Lentivirus vectors using human and simian immunodeficiency virus elements[J]. Journal of virology, 1999, 73(4): 2832-2840.
5. Kafri T, van Praag H, Ouyang L, et al. A packaging cell line for lentivirus vectors[J]. Journal of virology, 1999, 73(1): 576-584.

For research use only. Not intended for human and animal therapeutic or diagnostic use.

生产商：南京金斯瑞生物科技有限公司 江苏省南京市江宁区科学园雍熙路 28 号

Manufacturer: Nanjing GenScript Biotech Co., Ltd. No. 28 Yongxi Road, Jiangning District, Nanjing, Jiangsu, China