

Version 03

Update: 07/28/2023

# Product Manual

## Lentivirus Titer p24 ELISA Kit

Cat. No. L00938

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

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

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## I. DESCRIPTION

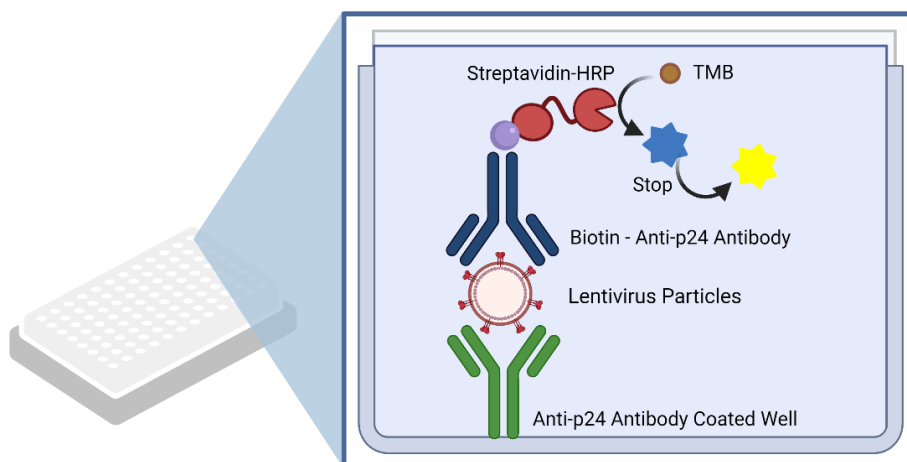
GenScript's Lentivirus Titer p24 ELISA kit (Cat. No. L00938) is a Sandwich ELISA designed for quantitatively measuring the physical titer of any HIV-1 based lentivirus in cell culture supernatant. For lentivirus purification and production, lentivirus titration is an essential characterization procedure for routine quality control and lot release, as well as for purification process optimization. This kit makes lentivirus titration easy, accurate and reproducible.

Lentivirus vector is derived from human immunodeficiency virus-1 (HIV-1). The vector, which contains a pseudotyped envelope with vesicular stomatitis virus envelope G (VSV-G) protein, has the ability to transfer gene(s) of interest into dividing and non-dividing cells. Many studies show that Lentiviral vectors have successfully delivered genes into neurons, lymphocytes, and macrophages. The vector is proven to be effective in transducing brain, liver, muscle, and retina *in vivo* without toxicity or immune responses. The vector is also used to integrate siRNA efficiently in a wide variety of cell lines.

## II. ASSAY PRINCIPLE

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 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

### 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 $\mu$ 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
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  $\mu$ 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 $\times$  Wash Solution** can be used as an alternative dilution buffer for samples and standards. The preparation instructions for the **1 $\times$  Wash Solution** is detailed in the PROTOCOL-Reagent Preparation-1 $\times$  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  $\mu$ L, 200  $\mu$ L, and 1000  $\mu$ L precision pipettes
- 10  $\mu$ L, 200  $\mu$ L, and 1000  $\mu$ L pipette tips
- Multichannel pipettes
- Disposable reagent reservoir
- Absorbent paper
- Laboratory timer
- Refrigerator
- Centrifuge
- 25  $\pm$  2  $^{\circ}$ C Incubator
- Rotary shaker

## 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 virus. 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 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 $^{\circ}$  - 25 $^{\circ}$ 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 reaction should be maintained at 25 $^{\circ}$ C.
11. Before opening the Standard Stock, quick 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 washing steps.

## 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 testing 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 refrigerator promptly after 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 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

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 prior to 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

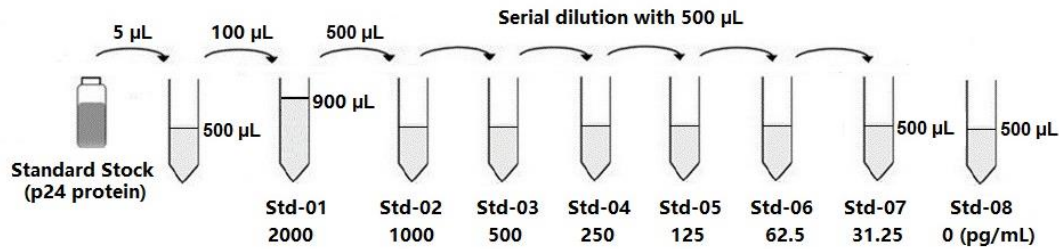
Centrifuge Standard Stock at 10,000 rpm for several seconds.

Dilute Standard Stock with fresh cell culture medium with a volume ratio of 1:1000. For example, mix 5 µL of Standard Stock with 500 µL of fresh cell culture medium first, and then dilute 100 µL of the mixture with 900 µL of fresh cell culture medium to make 1 mL of 2000 pg/mL Standard working solution with 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 standard curve.

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

Pipette 500  $\mu$ 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  $\mu$ 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 Capture Plate

	Standards		Samples									
	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	Std-1	Std-1	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
<b>B</b>	Std-2	Std-2	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
<b>C</b>	Std-3	Std-3	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
<b>D</b>	Std-4	Std-4	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
<b>E</b>	Std-5	Std-5	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
<b>F</b>	Std-6	Std-6	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
<b>G</b>	Std-7	Std-7	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
<b>H</b>	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  $\mu\text{L}$  of Lysis Solution to all the wells in the Capture Plate.
2. Add 100  $\mu\text{L}$  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  $\mu\text{L}$  of 1 $\times$  Wash Solution four times.
5. Tap inverted plate onto absorbent paper to remove residual liquid in the wells after the wash steps.

**Detection Antibody Incubation**

6. Add 100  $\mu\text{L}$  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  $\mu\text{L}$  of 1 $\times$  Wash Solution four times.
9. Tap inverted plate onto absorbent paper to remove residual liquid in the wells after wash steps.

**Enzyme Conjugate Incubation**

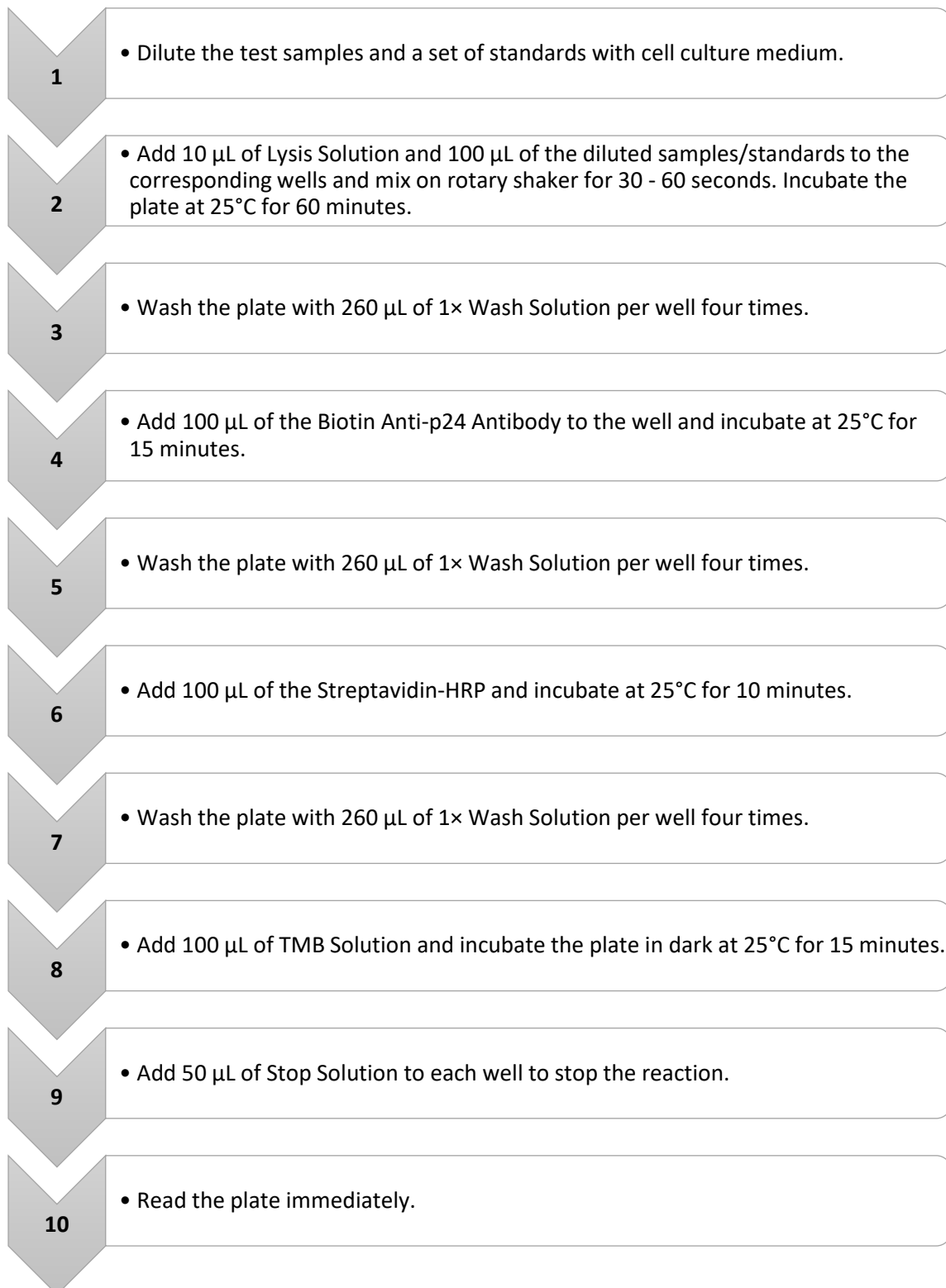
10. Add 100  $\mu\text{L}$  of Streptavidin-HRP to all the testing wells.
11. Cover the Plate with Plate Sealer and incubate at 25°C for 10 minutes.
12. Remove the Plate Sealer and wash the plate with 260  $\mu\text{L}$  of 1 $\times$  Wash Solution four times.
13. Tap inverted plate onto absorbent paper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

**Substrate Reaction and Absorbance Measurement**

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



## IX. ASSAY PROCEDURE SUMMARY



## X. INTERPRETATION OF RESULTS

- **Assay Validation**

To ensure the validity of results, the following criteria noted in the 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 create the standard curves. Choose a method with high Goodness of Fit (R<sup>2</sup>) 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 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)

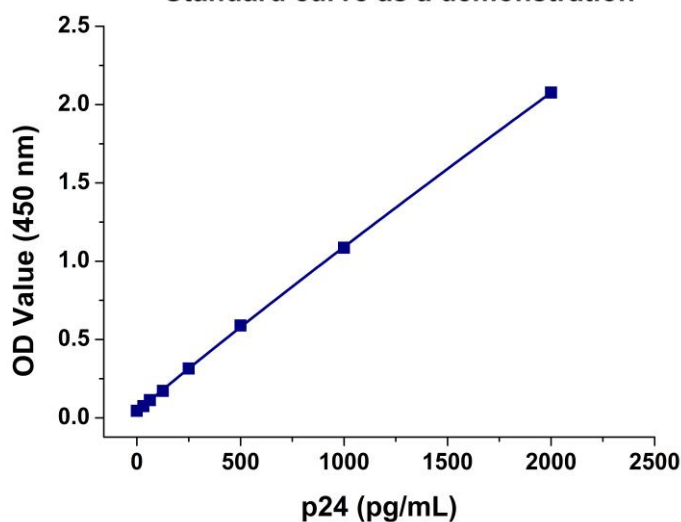
B = Hill's slope of the curve (i.e. this is related to the steepness of the curve at point c)

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

**Table 3.** Sample data for 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 =  $8 \times 10^{-5}$  pg of p24 or 1 ng p24 =*

$1.25 \times 10^7$  LPs. For reasonably packaged lentivirus vector, 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 concentration, using 3 lots of kits. From the assay, the intra-assay % CVs are less than 10%, 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

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

## XII. TROUBLESHOOTING

Problem	Probable Cause	Solution
<b>Poor Precision</b>	Wells are not washed or aspirated properly	Make sure the wash 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
<b>Weak/No Signal</b>	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 new Substrate from the same Lot
	Volumes of reagents are not correct	Repeat 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
<b>High Background</b>	Plate is not washed properly	Make sure the wash 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

### XIII. 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.

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