Product Manual

# QuickTiter™ HIV Lentivirus Quantitation Kit (HIV p24 ELISA)

## Catalog Numbers

| | |
|---|---|
| VPK-108-H | 96 assays |
| VPK-108-H-5 | 5 x 96 assays |

**FOR RESEARCH USE ONLY**
**Not for use in diagnostic procedures**

CELL BIOLABS, INC.
*Creating Solutions for Life Science Research*

## Introduction

Lentivirus vector based on the human immunodeficiency virus-1 (HIV-1) has become a promising vector for gene transfer studies. The advantageous feature of lentivirus vector is the ability of gene transfer and integration into dividing and non-dividing cells[1-2]. The pseudotyped envelope with vesicular stomatitis virus envelope G (VSV-G) protein broadens the target cell range. Lentiviral vectors have been shown to deliver genes to neurons, lymphocytes and macrophages, cell types that previous retrovirus vectors could not be used. Lentiviral vectors have also proven to be effective in transducing brain, liver, muscle, and retina *in vivo* without toxicity or immune responses. Recently, the lentivirus system is widely used to integrate siRNA efficiently in a wide variety of cell lines and primary cells both *in vitro* and *in vivo*.

Lentivirus particles are produced from 293T cells through transient transfection of 3 or 4 plasmids that encodes for the components of the virion. Viral medium containing viral particles produced by packaging cells within 48-72 hr can be harvested. To ensure that pseudoviral medium is viable, and to control the number of copies of integrated viral constructs per target cell, the viral titer needs to be determined before proceeding with transduction experiments. Viral titer can be determined by transduction of HT-1080 or HeLa cells, and followed by antibiotic selection of stable clones. However, it takes weeks to generate sizable stable cell colonies for counting and calculating the titer results.

Cell Biolabs' QuickTiter™ HIV Lentiviral Quantitation Kit (HIV p24 ELISA) is an enzyme immunoassay developed for detection and quantitation of the HIV-1 p24 core protein. A mouse monoclonal antibody to HIV-1 p24 is coated onto strip wells of microtiter plate. The quantity of HIV p24 antigen is determined by comparing its absorbance with that of known recombinant p24 antigen standard curve. The kit has a detection sensitivity limit of 1 ng/mL HIV p24, or about 10,000 to 100,000 TU/mL VSVG-pseudotyped lentivirus samples[3-5]. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples. The kit is suitable for both viral supernatant and purified virus.

The QuickTiter™ HIV Lentiviral Quantitation Kit is intended for research use only, and not for diagnostic applications.

## Assay Principle

An anti-HIV p24 monoclonal coating antibody is adsorbed onto a microtiter plate. p24 antigen present in the sample or standard binds to the antibodies adsorbed on the plate; a FITC-conjugated mouse anti-p24 antibody is added and binds to p24 antigen captured by the first antibody.

Following incubation and wash steps, a HRP-conjugated mouse anti-FITC antibody is added and binds to the FITC conjugated anti-p24. Following unbound HRP-conjugated mouse anti-FITC antibody is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

A colored product is formed in proportion to the amount of p24 antigen present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from recombinant HIV-1 p24 protein and sample p24 concentration is then determined.

## Related Products

1. LTV-100: 293LTV Cell Line
2. LTV-200: ViraDuctin™ Lentivirus Transduction Kit
3. LTV-300: GFP Lentivirus Control
4. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
5. VPK-112: QuickTiter™ Lentivirus Quantitation Kit

## Kit Components

### Box 1 (shipped at room temperature)

1. Anti-p24 Antibody Coated Plate (Part No. 310801): One strip well 96-well plate.
2. FITC-Conjugated Anti-p24 Monoclonal Antibody (Part No. 310810): One 20 µL vial.
3. HRP-Conjugated Anti-FITC Monoclonal Antibody (Part No. 310811): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. Triton X-100 Solution (Part No. 310805): One 15 mL bottle containing 5% Triton X-100 in TBS.
6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
8. Stop Solution (Part. No. 310808): One 12 mL bottle.

### Box 2 (shipped on blue ice packs)

1. Recombinant p24 Standard (Part No. 310809): One 100 µL vial of 10 µg/mL recombinant HIV1 p24 antigen in TBS plus BSA.

## Materials Not Supplied

1. Viral Sample: purified virus or unpurified viral supernatant
2. Cell Culture Centrifuge
3. 0.45 µm filter
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir
7. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

## Storage

Upon receiving, aliquot and store recombinant HIV-1 p24 Standard at -20ºC and avoid freeze/thaw. Store all other components at 4ºC.

## Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- FITC-Conjugated Anti-HIV1 p24 Monoclonal Antibody and HRP-Conjugated Anti-FITC Monoclonal Antibody: Immediately before use dilute the FITC-conjugated antibody 1:1000 and HRP-conjugated antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

## Safety Considerations

Remember that your samples contain infectious viruses before inactivation; you must follow the recommended NIH guidelines for all materials containing BSL-2 organisms.

## Preparation of Samples and Standards

### HIV p24 Standard Curve

1. Prepare a dilution series of recombinant HIV-1 p24 antigen in the concentration range of 100 ng/mL – 1 ng/mL by diluting the p24 stock solution in Assay Diluent (Table 1).

| Standard Tubes | Recombinant p24 Standard (µL) | Assay Diluent (µL) | p24 (ng/mL) |
|---|---|---|---|
| 1 | 10 | 990 | 100 |
| 2 | 500 of Tube #1 | 500 | 50 |
| 3 | 500 of Tube #2 | 500 | 25 |
| 4 | 500 of Tube #3 | 500 | 12.5 |
| 5 | 500 of Tube #4 | 500 | 6.25 |
| 6 | 500 of Tube #5 | 500 | 3.125 |
| 7 | 500 of Tube #6 | 500 | 1.5625 |
| 8 | 0 | 500 | 0 |

**Table 1. Preparation of p24 Antigen Standard**

2. Transfer 225µL of each dilution to a microcentrifuge tube containing 25 µL of Triton X-100 Solution. Perform the assay as described in Assay Instructions.

### II. Lentiviral Sample Dilution and Inactivation

1. (Optional) Dilute lentiviral supernatant in culture medium. Include culture medium as a negative control.
   *Note: Dilute 10 to 1000 folds for samples with infectious titer of $10^6$-$10^7$ TU/mL. For unknown samples, we recommend several dilutions for each sample.*
2. Transfer 225 µL of each sample to a microcentrifuge tube containing 25 µL of Triton X-100 Solution, Vortex well.
3. Incubate 30 minutes at 37ºC.

## Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Each lentiviral sample, HIV p24 standard, blank, and control medium should be assayed in duplicate.
3. Add 110 µL of inactivated sample or p24 antigen standard to anti-p24 antibody coated plate.
4. Cover with a Plate Cover and incubate at 4ºC overnight.
5. Remove Plate Cover and empty wells. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
6. Add 100 µL of the diluted FITC-Conjugated Anti-p24 Monoclonal Antibody to each well.
7. Cover with a Plate Cover and incubate at room temperature for 1 hour on an orbital shaker.
8. Remove Plate Cover and empty wells. Wash the strip wells 3 times according to step 5 above.
9. Add 100 µL of the diluted HRP-Conjugated Anti-FITC Monoclonal Antibody to all wells.
10. Cover with a Plate Cover and incubate at room temperature for 1 hour on an orbital shaker.
11. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to step 5 above. Proceed immediately to the next step.
12. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

    *Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.*
13. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
14. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

## Example of Results

The following figures demonstrate typical HIV p24 ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

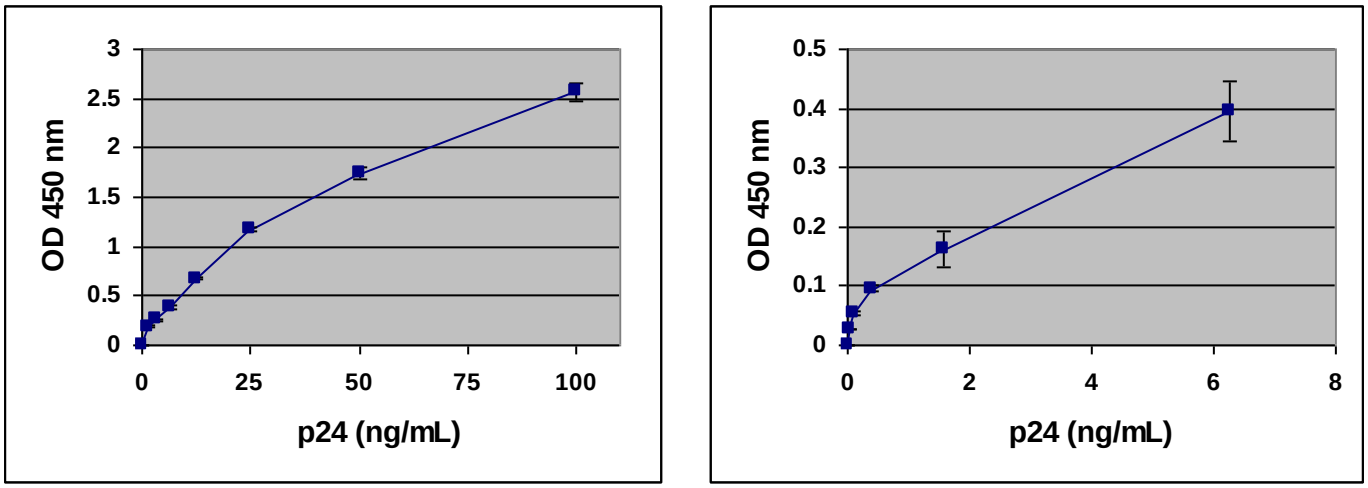


**Figure 1: HIV p24 ELISA Standard Curve.**

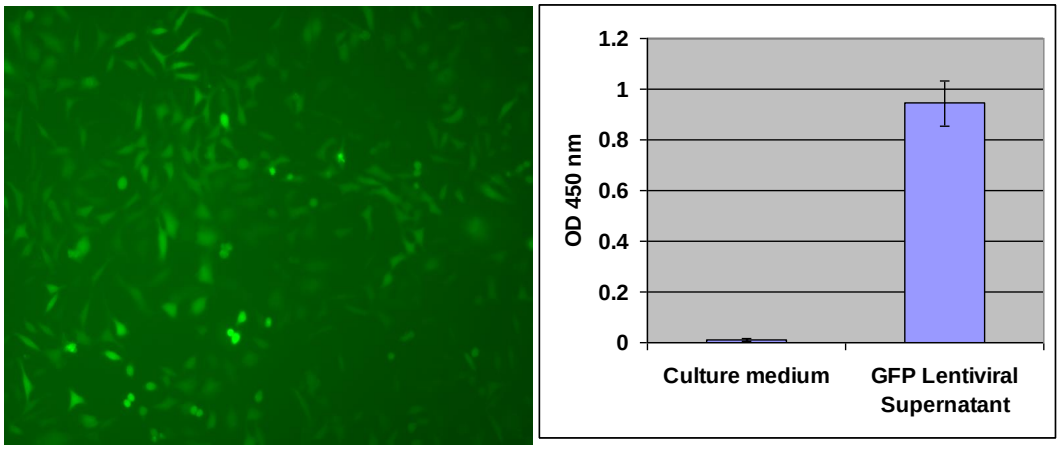


**Figure 2: p24 Titer of GFP Lentiviral Supernatant.** A GFP lentiviral construct was cotransfected with a packaging mix into 293AD cells (Cat.# AD-100). The conditioned medium was harvested 48 hrs after transfection. GFP expression was shown in HEK293 cells infected with the GFP lentiviral supernatant for 3 days. The p24 level in the diluted lentiviral supernatant (1:10 dilution) was determined as described in the assay instructions.

## Calculations

There are approximately 2000 molecules of p24 per Lentiviral Particle (LP), therefore,
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 a reasonably packaged lentivirus vector, 1 TU is about 100 to 1000 LP[3-5], therefore:

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

*Note: The calculated result is the lentivirus physical titer, p24 core protein level, and it is NOT the infectious titer (TU/mL). When the infectious titer is determined, the results vary among different target cell lines or transduction methods*[3-5].

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