Product Manual

QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)

Catalog Number

VPK-107	96 assays
VPK-107-5	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*.

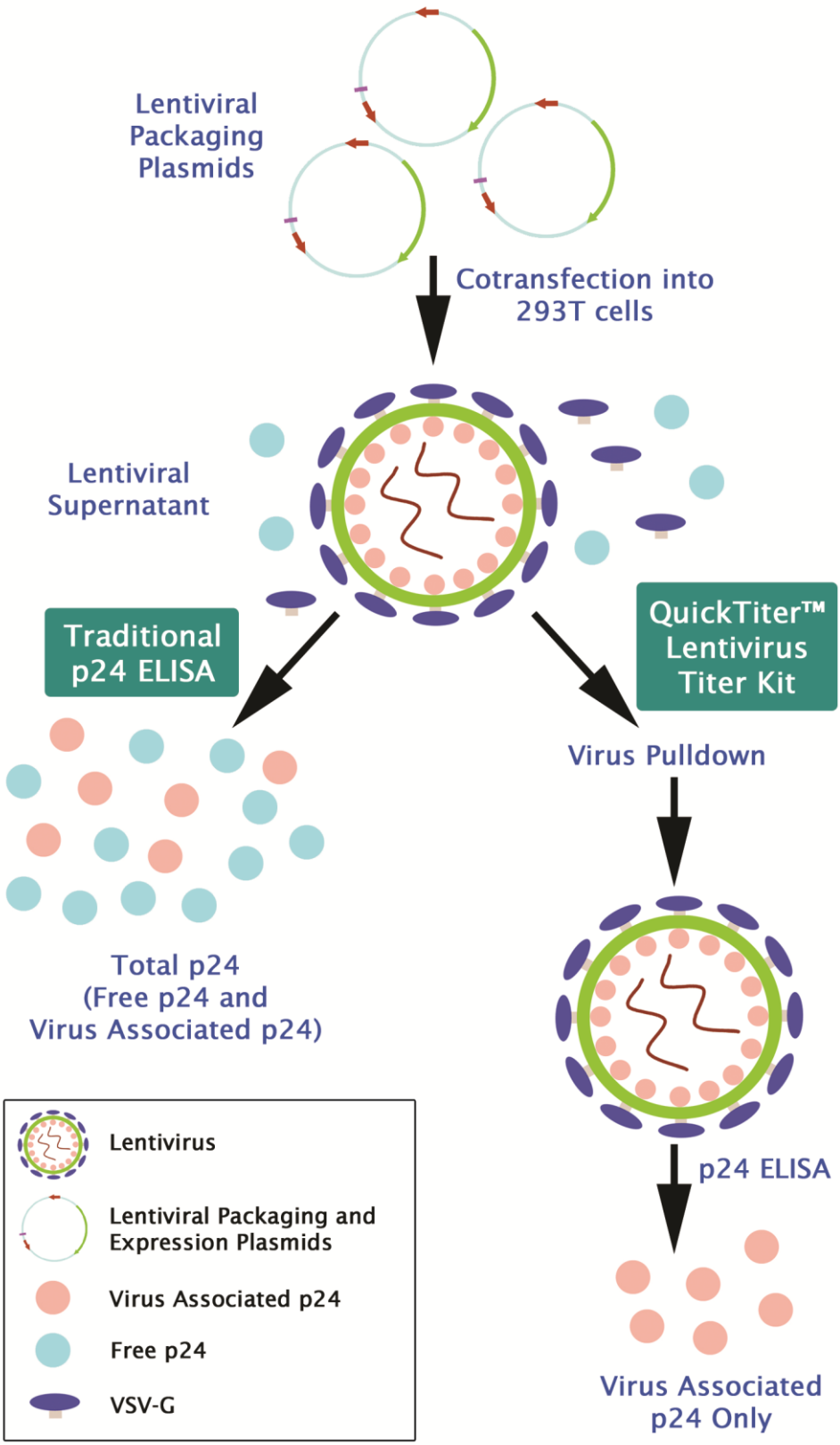
Lentivirus particles are produced from 293T cells through transient transfection of 3 or 4 plasmids that encodes for the components of the virion. Viral medium containing viral particles produced by packaging cells within 48-72 hr can be harvested. To ensure that pseudoviral medium is viable, and to 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.

HIV p24 ELISA has also been used in tittering lentiviral samples, but it detects both lentivirus associated p24 and free p24 generated by 293 T cells during transient transfection. Therefore, total p24 level (lentivirus p24 and free p24) can not be used to precisely determine the viral particles in lentivirus supernatant samples.

Cell Biolabs' QuickTiter™ Lentivirus Titer Kit (Lentivirus Associated HIV p24) is an enzyme immunoassay developed for detection and quantitation of the lentivirus associated HIV-1 p24 core protein only (See Assay Principle below). After forming complexes with ViraBind™ lentivirus reagents (patented technology), while free p24 remains in supernatant, the amount of lentivirus associated p24 is measured by a HIV p24 ELISA. The kit has 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 lentiviral samples.

QuickTiter™ Lentivirus Titer Kit (Lentivirus Associated HIV p24) provides an efficient system for rapid quantitation of lentivirus titer for both viral supernatant and purified virus.

Assay Principle



Related Products

1. LTV-100: 293LTV Cell Line
2. LTV-200: ViraDuctin™ Lentivirus Transduction Kit
3. LTV-300: GFP Lentivirus Control
4. VPK-108-H: QuickTiter™ Lentivirus Quantitation Kit (HIV p24 ELISA)
5. VPK-112: QuickTiter™ Lentivirus Quantitation Kit

Kit Components

Box 1 (shipped at room temperature)

1. ViraBind™ Lentivirus Reagent A (100X) (Part No. 310701): One 1.0 mL vial
2. ViraBind™ Lentivirus Reagent B (100X) (Part No. 310702): One 1.0 mL vial
3. Sample Diluent (Part No. 310703): One 50 mL bottle containing 0.5% Triton X-100
4. Anti-p24 Antibody Coated Plate (Part No. 310801): one strip well 96-well plate
5. FITC-Conjugated Anti-p24 Monoclonal Antibody (Part No. 310810): One 20 µL vial
6. HRP-Conjugated Anti-FITC Monoclonal Antibody (Part No. 310811): One 20 µL vial
7. Assay Diluent (Part No. 310804): One 50 mL bottle
8. 10X Wash Buffer (Part No. 310806): One 100 mL bottle
9. Substrate Solution (Part No. 310807): One 12 mL amber bottle
10. 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. Lentiviral Sample: purified virus or unpurified viral supernatant
2. Cell Culture Medium
3. Microcentrifuge
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 receipt, aliquot and store the Recombinant HIV-1 p24 Standard at -20ºC to avoid multiple freeze/thaw cycles. Store all other kit components at 4ºC.

Safety Considerations

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms.

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.

Preparation of 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 Sample Diluent (Table 1).

Standard Tubes	Recombinant p24 Standard (µL)	Sample 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	3.13
7	500 of Tube #6	500	1.56
8	0	500	0

Table 1. Preparation of p24 Antigen Standard

2. Vortex well and incubate 30 minutes at 37ºC.

Preparation and Inactivation of Lentiviral Samples

1. (Optional) Dilute lentiviral supernatant in fresh culture medium and keep the final volume of 1 mL for each sample. Include culture medium as a negative control.
   Note: For unknown samples, we recommend several dilutions for each sample.
2. Add 10 µL of ViraBind™ Lentivirus Reagent A (100X) to 1 mL of lentiviral sample, and mix by inverting. Immediately add 10 µL of ViraBind™ Lentivirus Reagent B (100X) and mix by inverting. Incubate 30 minutes at 37ºC.
3. Centrifuge 5 minutes at 12,000 rpm. Carefully remove the supernatant and dissolve the pellet in 250 µL of Sample Diluent. Vortex well and incubate 30 minutes at 37ºC to inactivate the viruses.

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 100 µL of inactivated lentiviral 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 QuickTiter™ Lentivirus Titer Kit (Lentivirus Associated HIV p24) results. One should use the data below for reference only. This data should not be used to interpret actual results.

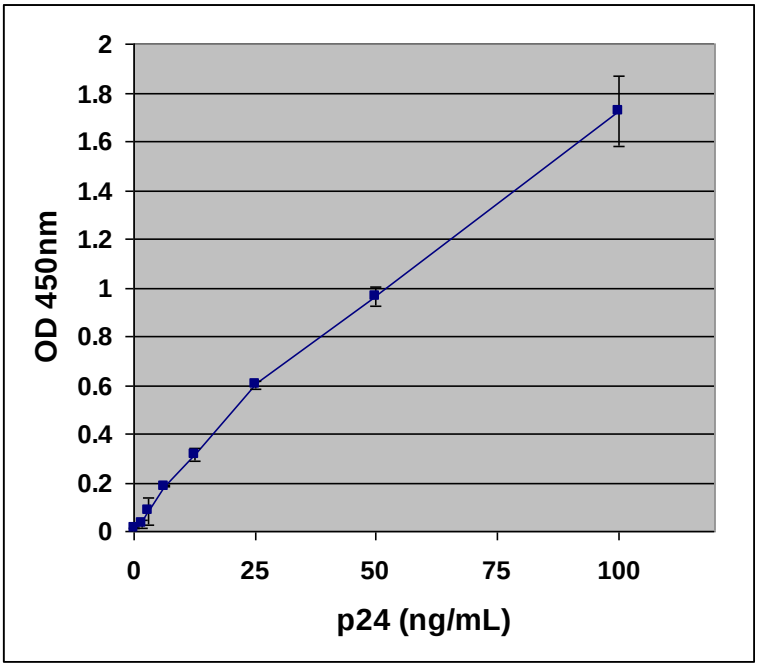


Figure 1: HIV p24 ELISA Standard Curve.

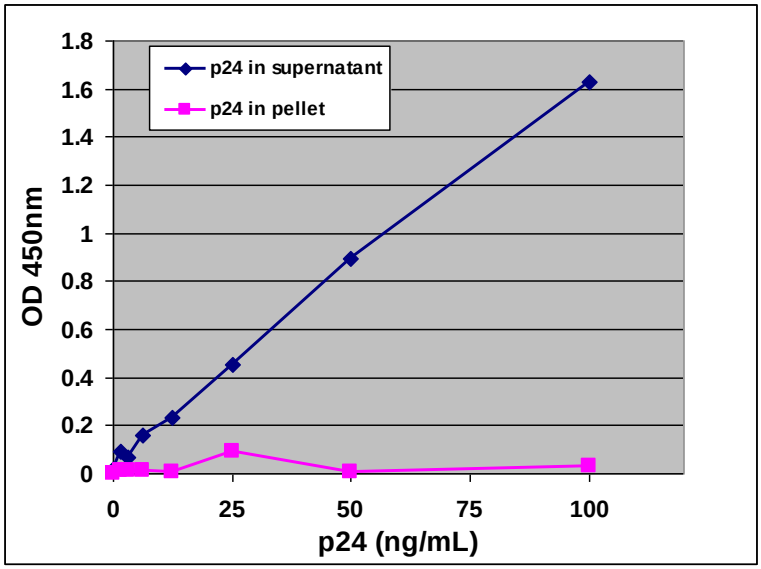


Figure 2: Free p24 does not complex with ViraBind™. Recombinant p24 diluted in culture medium was treated with ViraBind™ Lentivirus Reagents. The amount of p24 in supernatant and pellet was measured by p24 ELISA as described in Assay Protocol.

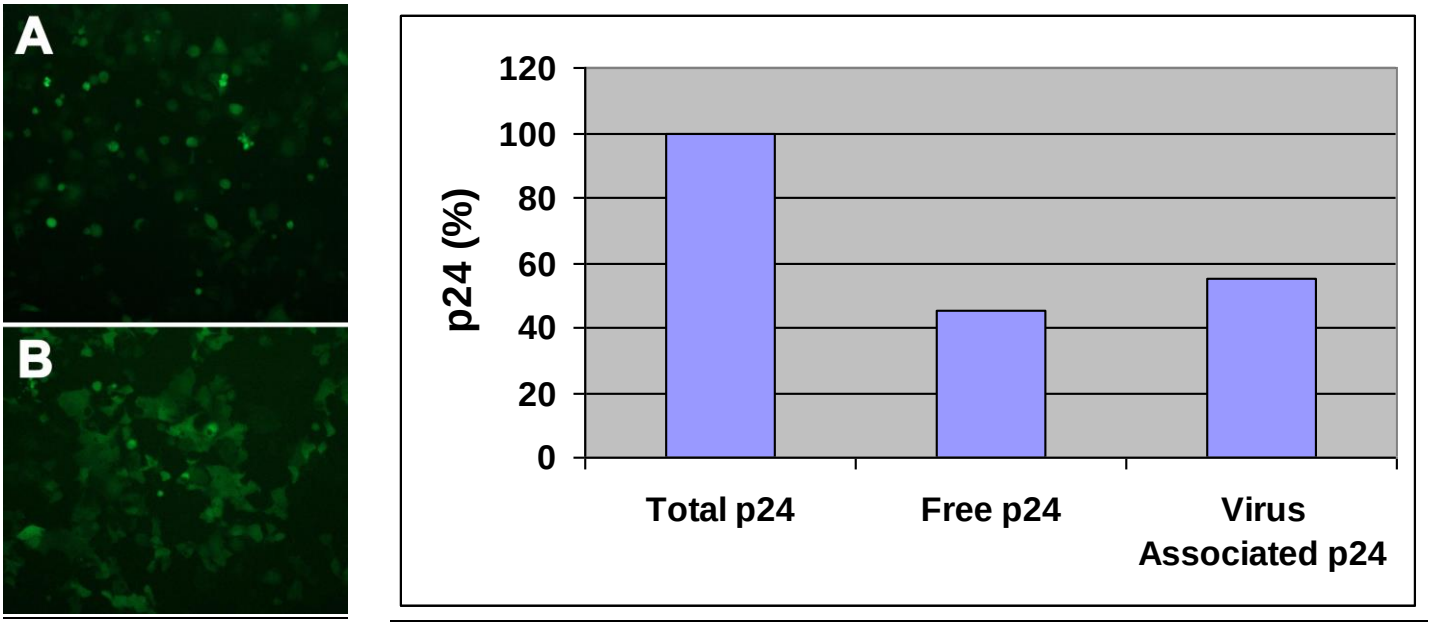


Figure 3: Virus Associated p24 Titer of GFP Lentiviral Supernatant. A GFP lentiviral construct was cotransfected with a packaging mix into 293LTV cells (Cat.# LTV-100). The conditioned medium was harvested 48 hrs after transfection. GFP expression was shown in HEK293 cells infected with the GFP lentiviral samples for 3 days (A: GFP lentiviral supernatant; B: ViraBind™ pellet). Free p24 and Virus Associated p24 were separated by ViraBind™ Lentivirus Reagents. The p24 level was determined as described in the assay instructions.

Calculation of Lentivirus Titer (VP/mL)

I. Determine Lentivirus Associated p24 Amount:

Based on p24 Standard curve, calculate the Lentivirus Associated p24 amount in the initial lentivirus sample.

p24 Titer (Virus associated p24, ng/mL) = p24 (ng/mL) x Dilution Factor x 0.25 mL/1.0 mL

II. Lentivirus Titer Calculation

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 = 8×10^{-5} pg of p24
or 1 ng p24 = 1.25×10^7 LPs

For 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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