QuickTiter™ FIV Lentivirus Quantitation Kit (FIV p24 ELISA)

Catalog Numbers

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>VPK-108-FIV-P24</td>
<td>96 tests</td>
</tr>
<tr>
<td>VPK-108-FIV-P24-5</td>
<td>5 x 96 tests</td>
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FOR RESEARCH USE ONLY
Not for use in diagnostic procedures
**Introduction**

Lentivirus vector based on the human immunodeficiency virus-1 (HIV-1) or feline immunodeficiency virus (FIV) 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. 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.

The most popular lentiviral expression system is HIV based. In spite of improved biosafety features, HIV-based lentiviral vectors still pose a potential biohazard risk due to the possible recombination with endogenous viral sequences to form a self-replicating HIV virus. A gene transfer system based on non-primate lentiviruses, such as feline immunodeficiency virus (FIV), may circumvent such concerns. Primates are not the natural host of FIV, and being a feline lentivirus, FIV has less sequence homology with HIV.

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™ FIV Lentiviral Quantitation Kit (FIV p24 ELISA) is an enzyme immunoassay developed for detection and quantitation of the FIV p24 core protein of lentiviral vector. An antibody to FIV p24 is coated onto strip wells of microtiter plate. The captured antigen is detected by another FIV p24 antibody on distinct epitope. Both capture and detection antibodies are specific to FIV p24 gag, they are negative against HIV p24, Feline Leukemia Virus, Feline Herpes Virus type 1, Feline Coronavirus and Feline Calicivirus. The quantity of FIV p24 antigen in lentiviral sample is determined by comparing its absorbance with that of known recombinant FIV p24 antigen standard curve. The kit has detection sensitivity limit of 2 ng/mL FIV p24, or about 10,000 to 60,000 TU/mL VSVG-pseudotyped FIV lentivirus samples. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and lentiviral samples.

QuickTiter™ FIV Lentiviral Quantitation Kit (FIV p24 ELISA) provides an efficient system for rapid quantitation of FIV lentivirus titer for both viral supernatant and purified virus.

**Related Products**

1. VPK-108-HIV-P24: QuickTiter™ Lentiviral Quantitation Kit (HIV p24 ELISA)
2. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
3. VPK-112: QuickTiter™ Lentivirus Quantitation Kit
4. VPK-200: ViraSafe™ Universal Lentivirus Expression System
5. LTV-100: 293LTV Cell Line
6. VPK-090: ViraBind™ Lentivirus Concentration and Purification Kit
7. VPK-095: ViraBind™ PLUS Lentivirus Concentration and Purification Kit
8. LTV-200: ViraDuctin™ Lentivirus Transduction Kit

**Test Principle**
An anti-FIV p24 coating antibody is adsorbed onto a microtiter plate. FIV p24 antigen present in the sample or standard binds to the antibodies adsorbed on the plate; a second biotin-conjugated anti-FIV p24 antibody is added and binds to p24 antigen captured by the first antibody. Both capture and detection antibodies are specific to FIV p24 gag, they are negative against HIV p24, Feline Leukemia Virus, Feline Herpes Virus type 1, Feline Coronavirus and Feline Calicivirus.

Following incubation and wash steps, Streptavidin-HRP is added and binds to the biotin conjugated anti-FIV p24. Following unbound Streptavidin-HRP 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 FIV 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 FIV p24 protein and sample FIV p24 concentration is then determined.

**Kit Components**

**Box 1 (shipped at room temperature)**
1. Anti-FIV p24 Antibody Coated Plate (Part No. 310814): one strip well 96-well plate.
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 FIV p24 Standard (Part No. 310812): One 50 µL vial of 10 µg/mL heat inactivated recombinant FIV p24 antigen in TBS plus BSA.

**Materials Not Supplied**
1. Lentiviral 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 wavelength)

**Storage**
Upon receiving, aliquot and store recombinant FIV p24 Standard at -20°C and avoid freeze/thaw. Store all other components at 4°C until their expiration dates.

**Preparation of Reagents**
- **1X Wash Buffer:** Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- **Biotinylated Anti-FIV p24 Antibody and Streptavidin-Enzyme Conjugate:** Immediately before use dilute the biotinylated antibody 1:1000 and Streptavidin-Enzyme conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

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

**Assay Sample Preparation**

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

<table>
<thead>
<tr>
<th>Standard Tubes</th>
<th>Recombinant FIV p24 Standard (µL)</th>
<th>Assay Diluent (µL)</th>
<th>FIV p24 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>990</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>500 of Tube #1</td>
<td>500</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>500 of Tube #2</td>
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<td>4</td>
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<td>7</td>
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</tr>
<tr>
<td>8</td>
<td>0</td>
<td>500</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1. Preparation of FIV 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 Instructions

1. Prepare and mix all reagents thoroughly before use.

2. Each lentiviral sample, FIV p24 standard, blank, and control medium should be assayed in duplicate.

3. Add 110 µL of inactivated sample or FIV p24 antigen standard to anti-FIV p24 antibody coated plate.

4. Cover with a Plate Cover and incubate at 37ºC for at least 4 hours or 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 biotinylated anti-FIV p24 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 Streptavidin-Enzyme Conjugate 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 wavelength.

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

![FIV p24 ELISA Standard Curve](image)

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

**Calculations**
There are approximately 2000 molecules of FIV p24 per Lentiviral Particle (LP), therefore, 1 LP contains:

\[
2000 \times 24 \times 10^3 / (6 \times 10^{23}) \text{ g of FIV p24} = 8 \times 10^{-5} \text{ pg of FIV p24}
\]

or 1 ng p24 = 1.25 x 10^7 LPs

For reasonably packaged lentivirus vector, 1 TU is about 100 to 1000 LP^{3-5}, therefore,

\[
10^6 \text{ TU/mL} = 10^{8-9} \text{ LP/mL} = 8 \text{ to 80 ng/mL of FIV p24}
\]
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\textsuperscript{3-5}.

References

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