

SIV p27

ANTIGEN CAPTURE ASSAY

Enzyme Immunoassay for the detection of Simian Immunodeficiency Virus
(SIV) p27 in tissue culture media

Catalog Numbers 5436 and 5450 (Bulk Format)

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FOR RESEARCH USE ONLY

SIV p27 ANTIGEN CAPTURE ASSAY

Note: The SIV p27 Antigen Capture Assay is for research use only and is not intended for diagnostic or clinical use.

INTRODUCTION

The Human Immunodeficiency Virus Type 1 (HIV-1) is the etiological agent of Acquired Immunodeficiency Syndrome (AIDS) in humans. The Simian Immunodeficiency Virus (SIV) is a similar virus which causes the AIDS like syndrome in rhesus macaques. The genomic structure of SIV is very similar to HIV-1. In fact, a virus, which is closely related to SIV, has been isolated from humans in West Africa and this has been named Human Immunodeficiency Virus Type-2 (HIV-2). The core of the SIV viral particle is made up of two strands of RNA and various proteins including the p27 core antigen. Since SIV p27 levels correlate well with viral load *in vitro*, measuring the amount of SIV p27 enables the quantification of the amount of virus present in tissue culture samples. The SIV p27 Antigen Capture Assay is a double antibody sandwich enzyme immunoassay that is used to calculate the concentration of SIV p27 in tissue culture samples. The assay's linear range is between 62.5 and 2000 pg / ml. Since the amino acid sequence of SIV p27 is well conserved among the various SIV isolates, this assay detects SIV p27 from various isolates with comparable sensitivity. The amino acid sequences of the HIV-2 p27 exhibit nearly 80-90% homology with SIV p27. Therefore, this assay cross reacts weakly with HIV-2 p27 but should not be used for its quantification. This assay does not cross react with HIV-1 p24 core antigen.

ASSAY OVERVIEW

Viral particles in test samples are disrupted with the Disruption Buffer, inactivating the virus and releasing the SIV p27 into solution to enable detection. The microtiter wells in a 96-well plate are coated with one murine monoclonal antibody that reacts with a unique epitope on SIV p27. When SIV p27 standard solutions or tissue culture samples are added to the wells, an immune complex forms with the plate-bound antibody and the SIV p27 in solution. Unbound materials are then thoroughly washed away. A solution containing peroxidase-conjugated mixture of monoclonal antibodies to SIV p27 is then added. The conjugate complexes with other epitopes on the captured SIV p27. After washing away the unbound conjugate, the peroxidase substrate is added. The enzyme-substrate reaction results in the substrate's blue color change. Upon adding the stop solution, the blue changes to yellow, which can be quantitatively measured by reading the absorbance at 450nm. The amount of SIV p27 in the standards or samples is therefore relative to the absorbance of the substrate. The concentration of SIV p27 in a sample can be interpolated from a standard curve or calculated through linear regression analysis.

PROVIDED COMPONENTS

Microelisa Plate	1 Plate (12 strips of 8 wells) Wells coated with murine monoclonal antibody to SIV p27. Plate is contained in a resealable foil bag with silica gel desiccant.
Disruption Buffer	1 Vial (10 ml) Contains 2.5% Triton-X-100 detergent and phosphate buffer.
Conjugate Solution	1 Vial (12 ml) Contains horseradish peroxidase-labeled, mouse monoclonal antibody to SIV p27.
Peroxidase Substrate	1 Vial (12 ml) A single component TMB-Hydrogen peroxide peroxidase substrate.
SIV p27 Standard	1 Vial (0.5 ml) Contains native SIV p27 10 ng/ml.
Wash Buffer (20X)	2 Vials (25 ml) Contains Phosphate Buffered Saline / Tween 20 concentrate. Dilute 20-fold before use.
Stop Solution	1 Vial (12 ml) Contains 2N sulfuric Acid. Warning – sulfuric acid is corrosive and can cause severe burns to skin and eyes.
Plate Sealers	5 Adhesive sheets

Store all kit components at 2-8°C. Do not freeze reagents.

ADDITIONAL REQUIRED MATERIALS

Distilled water
 Complete tissue culture medium
 Absorbent paper (paper towels)
 Timer
 V-bottomed reagent reservoirs
 Multichannel or single-channel micro-pipettors and pipet tips
 Incubator, 37° ± 0.5°C
 Microelisa plate washing system
 Microelisa plate reader (single wavelength 450 nm ± 5 nm)

ASSAY PROCEDURE

Preliminary Notes

1. For consistent results, bring all components and samples to room temperature (19 to 23°C) before use. Return the reagents to 2 to 8°C after use.
2. To prevent deterioration from condensation bring the foil pack containing the Microelisa Plate to room temperature (19-23°C) before opening. After opening, unused microelisa strips can be stored for up to 2 months, provided that the foil pack is resealed and the desiccant is not removed.
3. All reagents can be used only once.

Caution: Handle all reagents and samples as if capable of transmitting disease. The Conjugate Solution contains human derived material, and the SIV p27 Standard contains human and virus derived materials. Although, these reagents have been inactivated, there is no absolute assurance that such products cannot transmit infection. We recommend that all materials, samples and reagents be handled in accordance with Occupational Safety and Health Administration (OSHA) and the Centers for Disease Controls and Prevention (CDC) guidelines for working with SIV. Always follow Good Laboratory Practices (GLP) guidelines.

1. Always wear gloves and labcoats when handling kit reagents and samples.
2. Dispose of all materials and reagents used in this assay as hazardous waste.
3. The Stop Solution contains 2 N Sulfuric Acid and should never come in contact with Sodium Hypochlorite (bleach).

Caution: Stop Buffer contains 2N sulfuric acid, which can cause severe burns to the skin and eyes. Because sulfuric acid is corrosive, waste liquids containing sulfuric acid should be neutralized before disposal.

Sample Preparation

1. Tissue culture samples should be free of particulate matter. Centrifuge samples to remove cells and cell debris before use.
2. Samples must be free of sodium azide and microbial contamination.
3. Samples can be stored at -70°C before testing. However, avoid many freeze-thaw cycles, as they invalidate results.
4. Samples may need to be diluted in complete tissue culture media to be within the range of the assay.

Wash Procedure

1. If salt crystals are evident in the Wash Buffer (20 X), incubate @ 37°C until crystals dissolve.
2. Dilute 25 ml Wash Buffer (20X) in 475 ml distilled water. Diluted Wash Buffer will remain stable for 1 month @ $2-8^{\circ}\text{C}$.
3. If using an automated plate washing system, aspirate the well contents into a waste flask. Fill the wells with $300\ \mu\text{l}$ diluted Wash Buffer ($19-23^{\circ}\text{C}$), soak for 15 seconds, and then aspirate. Repeat wash procedure for a total of four washes.
4. After the last aspiration, invert the Microelisa Plate and tap firmly on absorbent paper (paper towel). Be careful not to dislodge any strips while tapping.

Alternatively: In absence of a plate washing system, drain plate and tap on absorbent paper (paper towel). Add diluted Wash Buffer and soak for 15-seconds. Drain and tap dry on clean absorbent paper after each soak.

Test Procedure

1. Add 25 µl of Disruption Buffer to each well of the Microelisa Plate to be used in the assay.
2. The assay is sensitive in the 62.5 through 2000 pg/ml linear range. Dilute the SIV p27 Standard in complete tissue culture media by the following method:

62.5 through 2000 pg/ml Linear Range			
SIV p27 Standard (pg/ml)	SIV p27 Standard Volume		Media Volume
2000	100 µl of 10 ng/ml	+	400 µl
1000	250 µl of 2000 pg/ml	+	250 µl
500	250 µl of 1000 pg/ml	+	250 µl
250	250 µl of 500 pg/ml	+	250 µl
125	250 µl of 250 pg/ml	+	250 µl
62.5	250 µl of 125 pg/ml	+	250 µl

3. Add 100 µl of each diluted SIV p27 Standard in duplicate to microelisa wells containing Disruption Buffer.
4. To serve as Negative Controls, add 100 µl of Complete Tissue Culture Media to 4 wells containing Disruption Buffer.
5. Add 100 µl of the prepared Test Samples to Microelisa Wells containing Disruption Buffer. It is recommended that these be performed in duplicate. It may be necessary to add several dilutions of the Test Samples to ensure they will be within the assay's range.
6. Incubate the Microelisa Plate, covered with a plate sealer, @ $37 \pm 0.5^{\circ}\text{C}$ for 60 ± 2 minutes.
7. Wash Microelisa Plate according to the previously stated Wash Procedure.
8. Add 100 µl of Conjugate Solution to each well.
9. Cover wells with a fresh Plate Sealer and incubate @ $37 \pm 0.5^{\circ}\text{C}$ for 60 ± 2 minutes.
10. Wash Microelisa Plate according to the previously stated Wash Procedure.
11. Add 100 µl of the prepared Peroxidase Substrate to each well. **Make Certain that the Peroxidase Substrate is @ room temperature before use.**
12. Incubate uncovered for 30 ± 1 minute @ room temperature ($19\text{-}23^{\circ}\text{C}$).

13. In the same order that the Peroxidase Substrate was added, add 100 μ l of the Stop Solution to each well. **Warning – Stop Solution contains 2N sulfuric acid, which is corrosive and can cause severe burns to skin and eyes.**

14. Read the Absorbance @ 450 nm in a Microelisa Plate Reader within 20 minutes.

Results

Qualification of Negative Controls Values

Negative Control absorbance values over 0.150 are not acceptable. If two or more values are above 0.150, then the run is invalid. Check washing procedure, incubation temperatures and component expiration dates, and repeat.

Qualification of SIV p27 Standards Values

The absorbance values of the 2000 pg/ml SIV p27 Standard should be >1.2 and < 2.4 . If the values are not within this range, then the run is invalid. Check washing procedure, incubation temperatures and component expiration dates, and repeat.

Qualification of Tissue Culture Sample Values

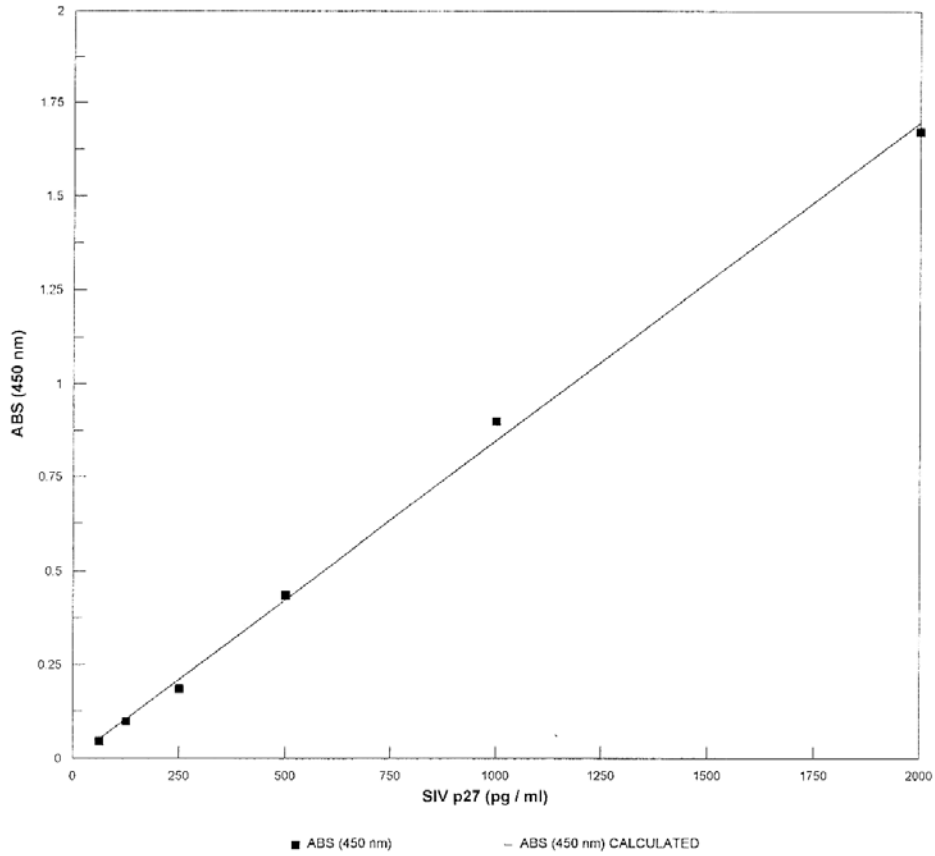
The absorbance values of the Test Samples should be between those of the lowest SIV p27 Standard (62.5 pg/ml) and the highest SIV p27 Standard (2000 pg/ml). If the absorbance of a Test Sample is below the absorbance of the lowest SIV p27 Standard, then the SIV p27 concentration is below the sensitivity of the assay. If the absorbance of a Test Sample is above the highest SIV p27 Standard, then the run must be repeated with a more diluted Test Sample to be within the assay's effective range.

Calculation of Test Sample SIV p27 Concentration

Calculate the mean absorbance for each SIV p27 Standard, Negative Control, and Test Sample. Subtract the background absorbance from the SIV p27 Standards and Test Samples by subtracting the mean absorbance of the Negative Controls from the mean absorbance of the SIV p27 Standards and Test Samples. Determine the SIV p27 concentration of each Test Sample by interpolating from a standard curve or by using linear regression analysis.

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Typical Standard Curve



	STANDARD pg/ml	A450 1	A450 2	A450 MEAN	A 450- BACKGROUND	pg/ml COMPUTED	A450- BACKGROUND COMPUTED
SIV p27	2000.0	1.798	1.717	1.758	1.673	1970.2	1.698
STANDARD	1000.0	0.998	0.972	0.985	0.901	1061.5	0.848
	500.0	0.530	0.510	0.520	0.436	514.5	0.423
	250.0	0.270	0.268	0.269	0.185	219.2	0.211
	125.0	0.181	0.182	0.182	0.097	116.3	0.104
	62.5	0.127	0.133	0.130	0.046	55.7	0.051
NEGATIVE CONTROL (BACKGROUND)	0	0.082	0.083	0.085	0.000		
	0	0.086	0.087				

Regression Output:

Constant	-0.002
Std Err of Y Est	0.033
R Squared	0.998
No. of Observations	6.000
Degrees of Freedom	4.000
X Coefficient(s)	0.001