

SIV p27 ANTIGEN CAPTURE ASSAY

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

Catalog #5436 and #5450



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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. The core of the SIV virion is made up of two strands of RNA and various proteins including the p27 core antigen. 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 has a linear range of 62.5 to 2000 pg/ml. Since the amino acid sequence of SIV p27 is well conserved among a number of SIV isolates, this assay detects SIV p27 from various isolates with comparable sensitivity. The amino acid sequences of HIV-2 p27 exhibits about 90% homology with SIV p27. Therefore, this assay cross reacts weakly with HIV-2 p27 but should not be used for its quantitation. This assay does not detect HIV-1 p24 core antigen.

ASSAY OVERVIEW

Test samples are mixed with disruption Buffer to inactivate virus and to release SIV p27 into solution to enable detection. The microtiter wells of a 96-well plate are coated with a murine monoclonal antibody that reacts with a unique epitope on SIV p27. When SIV p27 Standard solutions or Test 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. Conjugate Solution, containing a mixture of peroxidase-conjugated monoclonal antibodies to SIV p27, is then added. The conjugated antibodies complex with the captured SIV p27. After washing the wells to remove the unbound conjugated antibodies, Peroxidase Substrate is added to the wells. The enzyme-substrate reaction results in a blue color change. Upon adding Stop Solution, the blue color changes to yellow, and the absorbance is measured at 450 nm. There is a linear relationship between the absorbance at 450 nm and the amount of SIV p27 bound to the well. The concentration of SIV p27 in Test Samples can be determined from linear regression analysis of the standard curve.

PRODUCT WARRANTY

This product is for research use only and should not be used for clinical diagnostic purposes. ABL guarantees the quality and performance of all products used before the expiration date printed on the label. If a product is used according to manufacturer's instructions and fails to perform as described in the manual, please contact ABL to speak with a technical representative.

ASSAY COMPONENTS

Component	Cat# 5436 (1 plate)	Cat# 5450 (Bulk: 10 plates)
Microelisa Plate	1 plate	10 plates
Disruption Buffer	1 bottle of 10 ml	1 bottle of 100 ml
Conjugate Solution	1 bottle of 12 ml	1 bottle of 120 ml
Peroxidase Substrate	1 bottle of 12 ml	1 bottle of 120 ml
SIV p27 Standard	2 vials	10 vials
Wash Buffer (20X)	2 bottles of 25 ml	2 bottles of 250 ml
Stop Solution	1 bottle of 12 ml	4 bottles of 30 ml
Plate Sealers	5 adhesive sheets	30 adhesive sheets

Microelisa Plate	96 well plate coated with murine monoclonal antibody to SIV p27. Plate is contained in a resealable foil pouch with desiccant.
Disruption Buffer	Contains Triton® X-100 detergent and phosphate buffer.
Conjugate Solution	Contains horseradish peroxidase-labeled, mouse monoclonal antibodies to SIV p27.
Peroxidase Substrate	Contains hydrogen peroxide and tetramethylbenzidine in an acidic buffer.
SIV p27 Standard	Contains lyophilized SIV p27 at 10 ng/ml when reconstituted.
Wash Buffer (20X)	Contains Phosphate Buffered Saline / Tween 20® concentrate.
Stop Solution	Contains 2N sulfuric Acid. Warning – sulfuric acid is corrosive and can cause severe burns to skin and eyes.
Plate Sealers	Adhesive sheets.

Triton® X-100 is a registered trademark of The Dow Chemical Company. Tween 20® is a registered trademark of ICI Americas.

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

ADDITIONAL REQUIRED MATERIALS

Distilled water
 Complete tissue culture medium, containing 10% fetal bovine serum (FBS)
 Absorbent paper (paper towels)
 Timer
 V-bottomed reagent reservoirs
 Multichannel or single channel pipettes and pipette tips
 Incubator, 37° ± 0.5°C
 Microelisa plate washing system
 Microelisa plate reader (single wavelength 450 nm ± 5 nm)

ASSAY PROCEDURE

Preliminary Notes

1. The SIV p27 Antigen Capture Assay is for research use only and is not intended for diagnostic or clinical use.
2. For consistent results, bring all components and samples to room temperature (19- 23°C) before use. Return the reagents to 2-8°C after use.
3. Always bring the foil pouch 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 at 2-8°C, provided that the foil pack is resealed and the desiccant is not removed.
4. All reagents can be used only once.

Cautions

1. Handle all reagents and samples as if capable of transmitting disease. The Conjugate Solution contains human derived material, and the SIV p27 Standard contains 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 the Occupational Safety and Health Administration (OSHA) and the Centers for Disease Controls and Prevention (CDC) guidelines for working with HIV. Always follow Good Laboratory Practice (GLP) guidelines.
2. Always wear personal protective equipment, including gloves and lab coats, when handling kit reagents and samples.
3. Dispose of all materials, samples and reagents used in this assay as hazardous waste.
4. The Stop Solution 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.
5. The Stop Solution should never come in contact with Sodium Hypochlorite (bleach).

Sample Preparation

1. Tissue culture samples should be free of particulate matter. Centrifuge samples to remove cells and cell debris before use.
2. Test samples must be free of microbial contamination.
3. Test samples may be stored at -60° to -80° C before testing. However, avoid many freeze-thaw cycles, as they invalidate results.
4. Test samples may require dilution in complete tissue culture media (containing 10% FBS) to be within the range of the assay.

SIV p27 Standard Reconstitution

1. Warm 1 vial of SIV p27 Standard to room temperature ($19-23^{\circ}$ C).
2. Reconstitute SIV p27 Standard in complete tissue culture media (+ 10% FBS) in the volume specified on the vial's label. Gently swirl contents for 10 seconds and invert 5 times. Set at room temperature for 5 minutes and invert again 5 times. The concentration of SIV p27 is 10 ng/ml.
3. The reconstituted standard is stable for 1 week when stored at $2-8^{\circ}$ C and is stable for 2 months when stored frozen at -60° to -80° C. Reconstituted standard is stable after a single freeze-thaw. Avoid multiple freeze-thaw cycles.

Wash Procedure

1. If salt crystals are evident in the Wash Buffer (20 X), incubate at 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 at $2-8^{\circ}$ C.
3. If using an automated plate washing system, aspirate the well contents into a waste flask. Fill the wells with 300 μ l diluted Wash Buffer ($19-23^{\circ}$ 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. Dilute the reconstituted 10 ng/ml SIV p27 Standard in complete tissue culture media (containing 10% FBS) by the following method:

SIV p27 Standard Volume		Complete Tissue Culture Media Volume		Final Diluted SIV p27 Standard Concentration (pg/ml)
100 μl of 10 ng/ml	+	400 μl	=	2,000
250 μl of 2,000 pg/ml	+	250 μl	=	1,000
250 μl of 1,000 pg/ml	+	250 μl	=	500
250 μl of 500 pg/ml	+	250 μl	=	250
250 μl of 250 pg/ml	+	250 μl	=	125
250 μl of 125 pg/ml	+	250 μl	=	62.5

3. Add 100 μl of each diluted SIV p27 Standard to microelisa wells containing Disruption Buffer, in duplicate.
4. To serve as Negative Controls, add 100 μl of Complete Tissue Culture Media (containing 10% FBS) 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 results will be within the assay range.
6. Gently tap the side of the plate to mix, cover with a Plate Sealer and incubate at $37 \pm 0.5^\circ\text{C}$ 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 at $37 \pm 0.5^\circ\text{C}$ for 60 ± 2 minutes.
10. Wash Microelisa Plate according to the previously stated Wash Procedure.
11. Add 100 μl of Peroxidase Substrate to each well.
12. Incubate plate uncovered for 30 ± 1 minute at room temperature ($19\text{-}23^\circ\text{C}$).

13. In the same order that the Peroxidase Substrate was added, add 100 μ l of 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 plate absorbance at 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 times and temperatures and component expiration dates.

Qualification of SIV p27 Standards Values

The absorbance values of the 2,000 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 times and temperatures and component expiration dates.

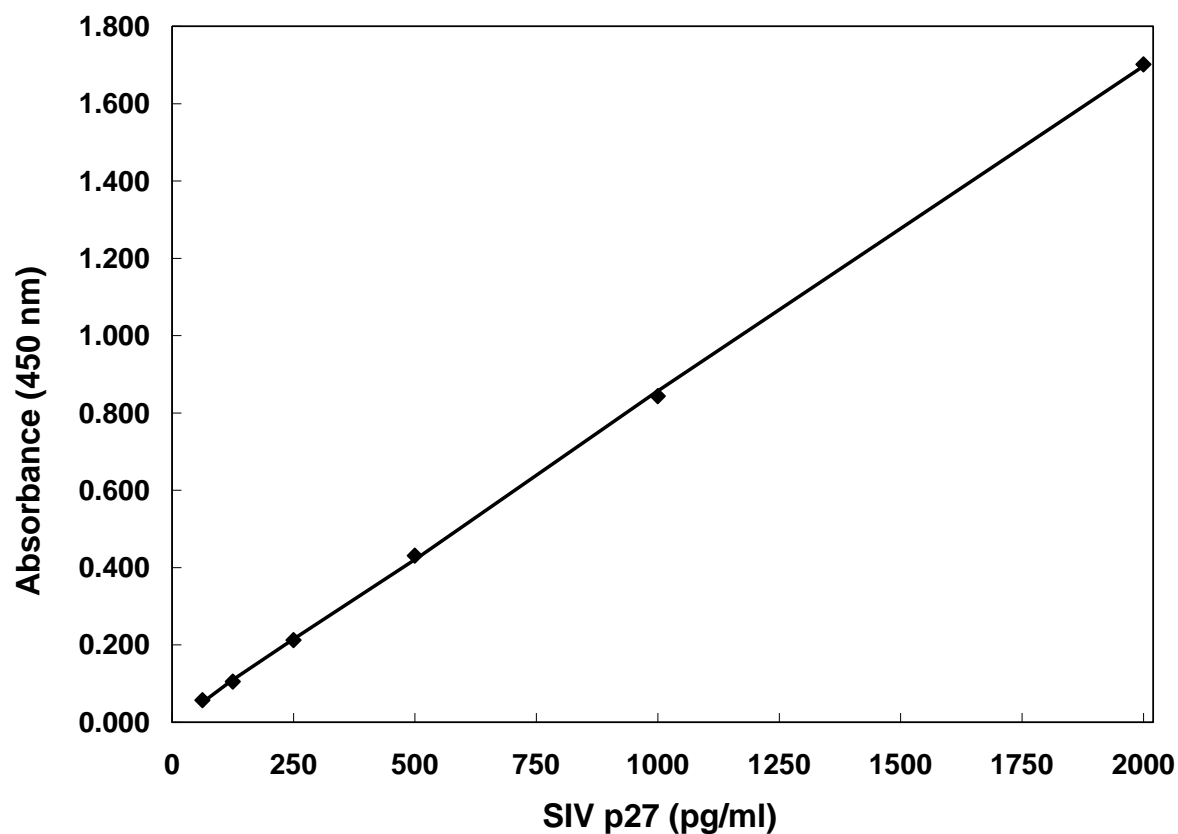
Qualification of Test Sample Values

To be considered valid, Test Sample absorbance values should be between those of the 62.5 pg/ml and 2,000 pg/ml SIV p27 Standards. If a Test Sample absorbance value is below the 62.5 pg/ml SIV p27 Standard value, then the SIV p27 concentration is below the sensitivity of the assay. If a Test Sample absorbance value is above the 2,000 pg/ml SIV p27 Standard value, 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

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

TYPICAL STANDARD CURVE



	STANDARD pg/ml	A450 1	A450 2	A450 MEAN	A450- BACKGROUND	pg/ml COMPUTED	A450- BACKGROUND COMPUTED
SIV p27	2000	1.790	1.714	1.752	1.702	2003	1.699
STANDARD	1000	0.930	0.858	0.894	0.844	992	0.850
	500	0.482	0.481	0.482	0.431	506	0.426
	250	0.267	0.258	0.263	0.212	248	0.213
	125	0.158	0.153	0.156	0.105	122	0.107
	62.5	0.108	0.107	0.108	0.057	65.8	0.054
NEGATIVE CONTROL (BACKGROUND)	0	0.049	0.053	0.051			
	0	0.052	0.052				

Regression Output:

Constant	0.001
Std Err of Y Est	0.005
R Squared	1.000
No. of Observations	6.000
Degrees of Freedom	4.000

X Coefficient(s)	0.001
Std Err of Coef.	0.00000

TROUBLESHOOTING GUIDE

Weak signal

Check incubation times and temperatures. If reagents are not allowed to reach room temperature prior to use or if the room temperature is cooler than acceptable range (19-23°C), the absorbance value may be unacceptably low. Make sure reagents have been warmed to room temperature prior to use.

If there are multiple plates in the same 37°C incubator, the plate may require more time to reach 37°. In such instances, incubation times may be increased up to an additional one half hour.

No signal

Check procedures used. Make sure proper diluent is used for samples and standards.

Check pH of wash buffer. Acceptable range is pH 7.4 ± 0.3 .

High background (two or more Negative Controls are above 0.150 absorbance)

Check washing procedure, incubation times and temperatures and component expiration dates. Be certain the medium used to dilute samples and standards is fresh.

Plate washers may vary in their efficiency to aspirate liquid from wells. Trace amounts of Conjugate Solution may react with the Peroxidase Substrate causing high background. Adding additional washing steps may reduce background.

Sample values are higher than the 2,000 pg/ml Standard

Dilute samples further so they are within the effective range of the assay.

Salt crystals in Disruption or Wash Buffer (20 X)

Reagents may have been exposed to temperatures below the optimum range (2-8°C). Warm to 37°C until crystals dissolve.

TECHNICAL ASSISTANCE

We value our customer's feedback as it allows us to keep improving our products. We encourage you to contact ABL if you have any questions or concerns: 800-225-5600 or info@ablinc.com.

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