HIV-1 p24
ANTIGEN CAPTURE ASSAY

Enzyme Immunoassay for the Detection of
Human Immunodeficiency Virus Type 1 (HIV-1) p24
in HIV-1 Tissue Culture and Lentiviral Vector Samples

Catalog #5421 and #5447

ABL inc.
ADVANCED BIOSCIENCE LABORATORIES

Version 7; 06/2021

ABL PRODUCTS AND SERVICES

ABL, Inc. is a contract development and manufacturing organization (CDMO) and contract research organization (CRO) providing GMP manufacturing and immunology solutions for gene therapies, oncolytics, vaccines and other immunotherapeutics. We specialize in immuno-oncology, infectious diseases, neurological diseases and chronic diseases. ABL has a global footprint, with facilities in the U.S. and Europe, offering integrated product development platforms for GMP virus and protein manufacture, bioanalytical services, biomarker identification and immunological testing. ABL also offers unique HIV-1, SIV and HTLV-I catalog products for customer R&D projects.

ABL's mission is to harness decades of pioneering science and manufacturing expertise to drive the development of innovative therapies and vaccines supporting the biopharmaceutical industry in their quest to improve public health.

Please visit us at www.ablinc.com and email us at info@ablinc.com to discuss how we can help advance your innovative therapies and vaccines with a revolutionary partnership.

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INTRODUCTION

Human Immunodeficiency Virus Type 1 (HIV-1) is a species of lentivirus that is the etiological agent of Acquired Immunodeficiency Syndrome (AIDS) in humans. Recombinant lentiviruses, based on a modified HIV-1 genome, are used as gene therapy vectors for the treatment for various human diseases. Lentiviral vectors exploit HIV's ability to reverse transcribe a RNA payload and integrate the foreign DNA into the host genome. The core of the HIV-1 virion and lentiviral vector are composed of two strands of RNA and various proteins including the HIV-1 p24 core antigen. The HIV-1 p24 Antigen Capture Assay is a double antibody sandwich enzyme immunoassay that is used to calculate the concentration of HIV-1 p24 in HIV-1 tissue culture and lentiviral vector samples. The assay has a linear range of 3.1 to 100 pg/ml. Since the amino acid sequence of HIV-1 p24 is well conserved among a number of HIV-1 isolates, this assay detects p24 from various isolates with comparable sensitivity. The sequence of Human Immunodeficiency Virus Type-2 (HIV-2) and Simian Immunodeficiency Virus (SIV) p27 core antigens exhibit about 60-70% homology with HIV-1 p24. This assay cross reacts weakly with HIV-2 and SIV p27, but should not be used for quantitation. There is no cross reactivity with Human T-Cell Leukemia Virus Types I and II (HTLV I and II) p24.

ASSAY OVERVIEW

Test Samples are mixed with Disruption Buffer to inactivate the virus and to release HIV-1 p24 into solution to enable detection. The microtiter wells of a 96-well plate are coated with two murine monoclonal antibodies that react with unique epitopes on HIV-1 p24. When HIV-1 p24 Standard solutions or tissue culture Test Samples are added to the wells, an immune complex forms with the plate-bound antibodies and the p24 in solution. Unbound materials are then thoroughly washed away. Conjugate Solution, containing peroxidase-conjugated human anti-p24 polyclonal antibodies, is then added. The conjugated antibodies complex with the captured HIV-1 p24. 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 HIV-1 p24 bound to the well. The concentration of HIV-1 p24 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# 5421 (1 plate)	Cat# 5447 (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
HIV-1 p24 Standard	1 vial of 0.5 ml	5 vials of 0.5 ml
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 antibodies to HIV-1 p24. 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, immunoaffinity purified, human antibodies to HIV-1 p24.

Peroxidase Substrate — Contains buffered hydrogen peroxide and tetramethylbenzidine.

HIV-1 p24 Standard — Contains purified native HIV-1_{IIIB} p24 at 1 ng/ml.

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 pipette 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 HIV-1 p24 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 HIV-1 p24 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 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 Test Samples should be free of particulate matter. Centrifuge Test 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 multiple freeze-thaw cycles, as this may invalidate results.

4. Test Samples may require dilution in complete tissue culture media (containing 10% FBS) to be within the range of the assay.

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°C.

3. If using an automated plate washing system, aspirate the well contents into a waste flask. Fill the wells with 300 µl diluted Wash Buffer (19-23°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 an automated plate washing system, drain plate and tap on absorbent paper (paper towel). Manually add approximately 300 µl/well diluted Wash Buffer and soak for 15-seconds. Drain and tap dry on clean absorbent paper after each soak. Repeat wash procedure for a total of four washes.

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 HIV-1 p24 Standard in Complete Tissue Culture Media (containing 10% FBS) by the following method:

HIV-1 p24 Standard Volume		Complete Tissue Culture Media Volume		Final Diluted HIV-1 p24 Standard Concentration (pg/ml)
50 µl of 1 ng/ml	+	450 µl	=	100
250 µl of 100 pg/ml	+	250 µl	=	50
250 µl of 50 pg/ml	+	250 µl	=	25
250 µl of 25 pg/ml	+	250 µl	=	12.5
250 µl of 12.5 pg/ml	+	250 µl	=	6.3
250 µl of 6.3 pg/ml	+	250 µl	=	3.1

3. Add 100 µl of each diluted HIV-1 p24 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 ± 0.5°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 ± 0.5°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 (19-23°C).
13. In the same order that the Peroxidase Substrate was added, add 100 µl 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 0.120 are not acceptable. If two or more values are above 0.120, then the run is invalid. Check washing procedure, incubation times and temperatures and component expiration dates.

Qualification of HIV-1 p24 Standards Values
The absorbance values of the 100 pg/ml HIV-1 p24 Standard should be >1.2 and < 2.2. If values are not within this range, then the run is invalid. Check washing procedure, incubation times and temperatures and component expiration dates.

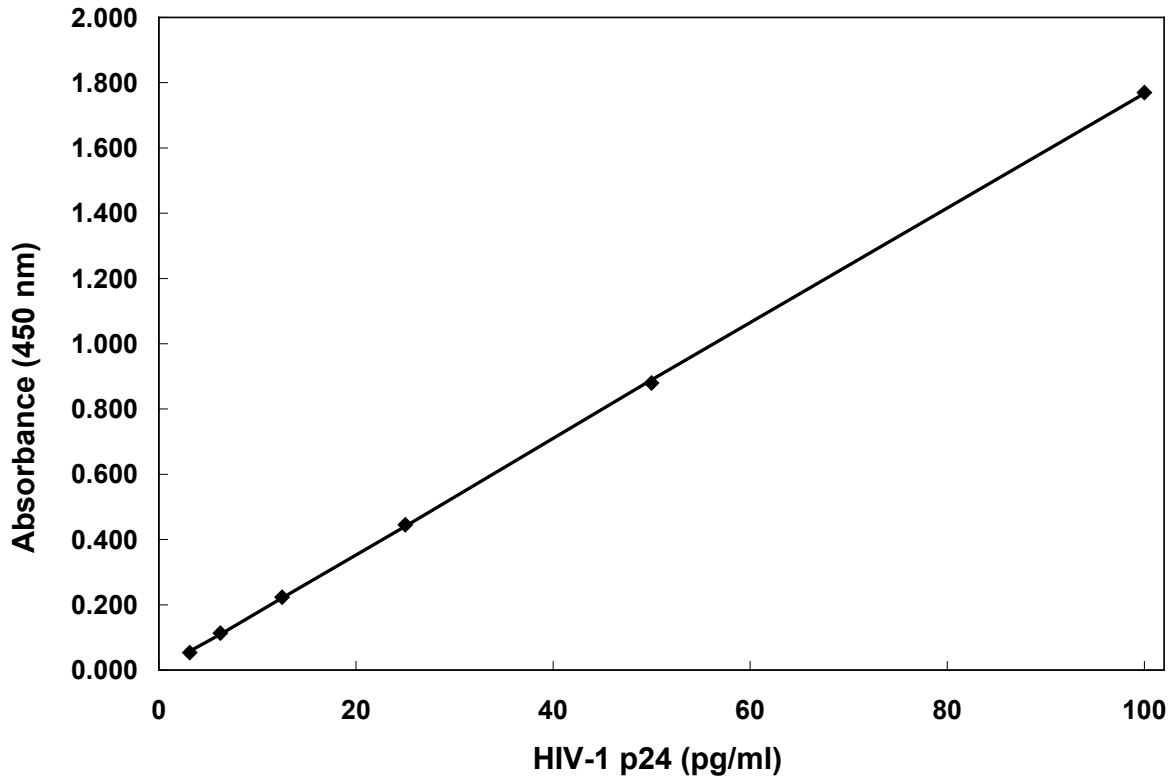
Qualification of Test Sample Values
To be considered valid, Test Sample absorbance values should be between those of the 3.1 pg/ml and the 100 pg/ml HIV-1 p24 Standards. If a Test Sample absorbance value is below the 3.1 pg/ml HIV-1 p24 Standard value, then the p24 concentration is below the sensitivity of the assay. If a Test Sample absorbance value is above the 100 pg/ml HIV-1 p24 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 HIV-1 p24 Concentration

1. Calculate the mean absorbance for each HIV-1 p24 Standard, Negative Control, and Test Sample. To subtract the background, subtract the mean absorbance of the Negative Controls from the mean absorbance of the HIV-1 p24 Standards and Test Samples.

2. Determine the HIV-1 p24 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
HIV-1 p24	100	1.840	1.819	1.830	1.770	100	1.768
STANDARD	50	0.944	0.935	0.940	0.880	50	0.885
	25	0.512	0.497	0.505	0.445	25	0.443
	12.5	0.284	0.281	0.283	0.223	12.6	0.222
	6.3	0.174	0.171	0.173	0.113	6.3	0.111
	3.1	0.114	0.112	0.113	0.054	3.0	0.056
NEGATIVE CONTROL	0	0.057	0.053	0.060			
(BACKGROUND)	0	0.064	0.062				

Regression Output:

Constant		0.001
Std Err of Y Est		0.003
R Squared		1.000
No. of Observations		6.000
Degrees of Freedom		4.000
X Coefficient(s)	0.018	
Std Err of Coef.	0.00004	

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), absorbance values 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.

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.

ABL, Inc.
9800 Medical Center Drive, Building D
Rockville, MD 20850

800-225-5600
info@ablinc.com
www.ablinc.com