

# **HIV-1 p24**

## **ANTIGEN CAPTURE ASSAY**

Enzyme Immunoassay for the detection of Human Immunodeficiency Virus  
Type 1 (HIV-1) p24 in tissue culture media.

**Catalog Numbers 5421 and 5447 (Bulk Format)**

 Advanced BioScience Laboratories, Inc.

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5510 Nicholson Lane  
Kensington, MD 20895-1078  
301-881-5600

FOR RESEARCH USE ONLY

# HIV-1 p24 ANTIGEN CAPTURE ASSAY

**Note:** The HIV-1 p24 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 core of the viral particle is made up of two strands of RNA and various proteins including the p24 core antigen. Since p24 levels correlate well with viral load *in vitro*, measuring the amount of p24 enables the quantification of the amount of virus present in tissue culture samples. 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 tissue culture samples. The assay's linear range is between 3.1 and 100 pg / ml. Since the amino acid sequence of p24 is well conserved among the various HIV-1 isolates, this assay detects p24 from various isolates with comparable sensitivity. The genetic code of the Human Immunodeficiency Virus Type-2 (HIV-2) and Simian Immunodeficiency Virus (SIV) p27 core antigens exhibit about 60% homology with HIV-1 p24. Therefore, this assay cross reacts weakly with HIV-2 and SIV p27, but should not be used for quantification. There is no cross reactivity with Human T-Cell Leukemia Virus Types I and II (HTLV I and II) p24.

## ASSAY OVERVIEW

Viral particles in Test Samples are disrupted with the Disruption Buffer, inactivating the virus and releasing the p24 into solution to enable detection. The microtiter wells in a 96-well plate are coated with two murine monoclonal antibodies that react with unique epitopes on HIV-1 p24. When 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. The Conjugate Solution, containing peroxidase-conjugated human anti-p24 polyclonal antibodies is then added. The conjugated antibodies complex with other epitopes on the captured p24. After washing away the unbound Conjugate Solution, 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 p24 in the p24 Standards or Test Samples is therefore relative of the extent of the color change. The concentration of p24 in a Test Sample can be interpolated from a graph of the absorbance versus concentration of a p24 Standard dilution series or calculated through linear regression analysis.

## PROVIDED COMPONENTS

Microelisa Plate	1 Plate (12 strips of 8 wells) Wells coated with murine monoclonal antibodies to HIV-1 p24. 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, immunoaffinity purified, Human antibodies to HIV-1 p24.
Peroxidase Substrate	1 Vial (12 ml) Contains H <sub>2</sub> O <sub>2</sub> and tetramethylbenzidine in an acidic buffer..
p24 Standard	1 Vial (2 ml) Contains HPLC purified native HIV-1 <sub>IIIIB</sub> p24 @ 1 ng/ml. Concentration determined by amino acid composition analysis.
Wash Buffer (20X)	2 Bottles (25 ml) Contains Phosphate Buffered Saline / Tween 20 concentrate. Dilute 20-fold before use.
Stop Solution	1 Bottle (12 ml) Contains 2N sulfuric Acid. <b>Warning – sulfuric acid is corrosive and can cause severe burns to skin and eyes.</b>
Plate Sealers	5 Adhesive sheets

**Store all kit components at 4°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 4°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 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 Occupational Safety and Health Administration (OSHA) and the Centers for Disease Controls and Prevention (CDC) guidelines for working with HIV. 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 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 sodium azide and microbial contamination.
3. Test Samples can be stored at  $-70^{\circ}\text{C}$  before testing. However, avoid many freeze-thaw cycles, as they invalidate results.
4. Test 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 @  $37^{\circ}\text{C}$  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  $\mu\text{l}$  of Disruption Buffer to each well of the Microelisa Plate to be used in the assay.

2. The assay is sensitive in the 3.1 through 100 pg/ml linear range. Dilute the p24 Standard in Complete Tissue Culture Media by the following method:

p24 Standard (pg/ml)	p24 Standard Volume		Media Volume
100	50 µl of 1 ng/ml	+	450 µl
50	250 µl of 100 pg/ml	+	250 µl
25	250 µl of 50 pg/ml	+	250 µl
12.5	250 µl of 25 pg/ml	+	250 µl
6.25	250 µl of 12.5 pg/ml	+	250 µl
3.1	250 µl of 6.25 pg/ml	+	250 µl

3. Add 100 µl of each diluted p24 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. Gently tap the side of the plate to mix.
6. Incubate the Microelisa Plate, covered with a Plate Sealer, @  $37 \pm 0.5^{\circ}\text{C}$  for  $60 \pm 2$  minutes. *Under some circumstances, incubation of up to 90 minutes may be used to increase Absorbance values at step 14 (See section "Results: Qualification of p24 Standards Values").*
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  $60 \pm 2$  minutes.
10. Wash Microelisa Plate according to the previously stated Wash Procedure.
11. Add 100 µl of the Peroxidase Substrate to each well. **Make certain that the Peroxidase Substrate is at room temperature before adding.**
12. Incubate uncovered for  $30 \pm 1$  minute @ room temperature ( $19\text{-}23^{\circ}\text{C}$ ).
13. In the same order that the Peroxidase Substrate was added, add 100 µl 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 0.120 are not acceptable. If two or more values are above 0.120, then the run is invalid. Check washing procedure, incubation temperatures and component expiration dates, and repeat.

### **Qualification of p24 Standards Values**

The absorbance values of the 100 pg/ml p24 Standard should be >1.200 and < 2.200. If values are not within this range, then the run is invalid. Check washing procedure, incubation temperatures and component expiration dates, and repeat. *For steps 1 through 6 of the Test Procedure, if reagents are not allowed to reach room temperature prior to use, if room temperatures are cool, or if there are several Microelisa Plates incubating at 37°C in the same incubator, the absorbance values at step 14 may be unacceptably low. In such instances, the p24 Standards and Test Samples on the Microelisa Plate may require a longer incubation to reach the 37°C necessary to facilitate optimum capture of p24 antigens on the plate. Acceptable absorbance values may be obtained by extending the step 6 incubation time from 60 to 90 minutes.*

### **Qualification of Tissue Culture Sample Values**

The absorbance values of the Test Samples should be between those of the 3.1 pg/ml and the 100 pg/ml p24 Standards. If a Test Sample's absorbance value is below the 3.1 pg/ml p24 Standard's value, then the p24 concentration is below the sensitivity of the assay. If a Test Sample's absorbance value is above the 100 pg/ml p24 Standard's 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 p24 Concentration**

1. Calculate the mean absorbance for each p24 Standard, Negative Control, and Test Sample. Subtract the background absorbance from the p24 Standards and Test Samples by subtracting the mean absorbance of the Negative Controls from the mean absorbance of the 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.