HIV-1 gp120
ANTIGEN CAPTURE ASSAY

Enzyme Immunoassay for the detection of Human Immunodeficiency Virus Type 1 (HIV-1) gp120 in tissue culture media.

Catalog # 5429

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

FOR RESEARCH USE ONLY

HIV-1 gp120 ANTIGEN CAPTURE ASSAY

Note: The HIV-1 gp120 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 envelope of HIV-1 is made up of external envelope glycoprotein gp120 and transmembrane glycoprotein gp41. The loss of these proteins leads to the loss of infectivity of the virus. Unlike the core antigen p24, the gp120 is highly variable among different isolates of HIV-1. Thus the gp120 exhibits a number of highly type specific antigenic determinants. The HIV-1 gp120 Antigen Capture Assay is a double antibody sandwich enzyme immunoassay that is used to calculate the concentration of HIV-1 gp120 in tissue culture samples. The assay's linear range is between 63 and 2000 pg / ml**. The assay is applicable for only Clade B isolates and detects the X4 or R5 isolates with comparable sensitivity. However the assay is applicable only for Clade B isolates and detects poorly the gp120 of other Clades. For example the sensitivity is 10 fold lower for Clade E gp120 and 100 fold lower for some of the Clade C gp120s.**

ASSAY OVERVIEW

Viral particles in Test Samples are disrupted with the Disruption Buffer, inactivating the virus and releasing the gp120 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 gp120. When gp120 Standard Solutions or tissue culture Test Samples are added to the wells, an immune complex forms with the plate-bound antibodies and the gp120 in solution. Unbound materials are then thoroughly washed away. The Conjugate Solution, containing peroxidase-conjugated human anti-gp120 polyclonal antibodies is then added. The conjugated antibodies complex with other epitopes on the captured gp120. 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 gp120 in the gp120 Standards or Test Samples is therefore relative of the extent of the color change. The concentration of gp120 in a Test Sample can be interpolated from a graph of the absorbance versus concentration of a gp120 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 gp120. 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 gp120.
Peroxidase Substrate	1 Vial (12 ml) Contains H_2O_2 and tetramethylbenzidine in an acidic buffer..
gp120 Standard	Two vials of lyophilized, purified HIV-1 gp120 standard. Reconstitute each vial with 500 µl of complete RPMI1640 medium. The concentration is 10000 picograms per ml.
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. **Warning – sulfuric acid is corrosive and can cause severe burns to skin and eyes.**
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 gp120 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 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.

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° 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°C until crystals dissolve.

2. Dilute 25 ml Wash Buffer (20X) in 475 ml distilled water. Mix thoroughly and check the pH. The pH should be 7.2 ± 0.2. Adjust with 2 N NaOH or 2 N HCl if needed. Diluted Wash Buffer will remain stable for 1 month @ 4°C.

3. If using a 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, 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), then add diluted Wash Buffer. Following each 15-second soak, drain and tap dry on clean absorbent paper.

Test Procedure

Add 25 µl of Disruption Buffer to each well of the Microelisa Plate to be used in the assay.

1. The assay is sensitive in the 63 through 2000 pg/ml linear range. Reconstitute the lyophilized gp120 Standard in 500 µl Complete Tissue Culture Medium (containing 10% Fetal Bovine Serum), to make 10000 pg/ml. Dilute the reconstituted gp120 Standard by the following method:

gp120 Standard (pg/ml)	**gp120 Standard Volume**		**Media Volume**
2000	100 µl of 10000 pg/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 gp120 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 ± 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 @ 37 ± 0.5°C for 60 ± 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 ± 1 minute @ room temperature (19-23°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 0.200 are not acceptable. If two or more values are above 0.200, 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 63 pg/ml and the 2000 pg/ml gp120 Standards. If a Test Sample's absorbance value is below the 63 pg/ml gp120 Standard's value, then the gp120 concentration is below the sensitivity of the assay. If a Test Sample's absorbance value is above the 2000 pg/ml gp120 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 gp120 Concentration

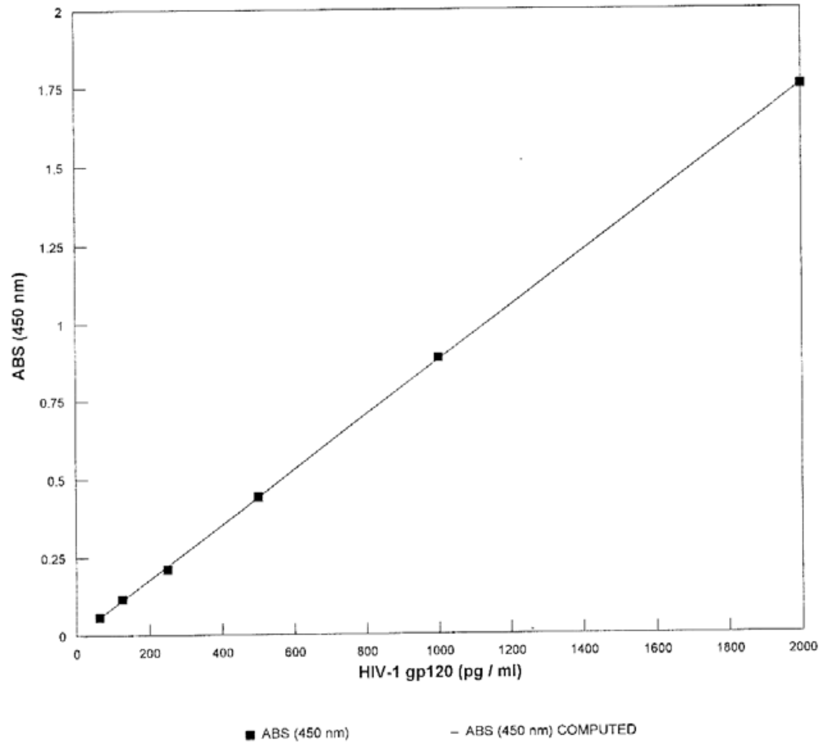
1. Calculate the mean absorbance for each gp120 Standard, Negative Control, and Test Sample. Subtract the background absorbance from the gp120 Standards and Test Samples by subtracting the mean absorbance of the Negative Controls from the mean absorbance of the gp120 Standards and Test Samples.

2. Determine the HIV-1 gp120 concentration of each Test Sample by interpolating from a standard curve or by using linear regression analysis.

The absorbance values and the standard curve derived from a typical assay follows:

HIV-1 gp120 ANTIGEN CAPTURE ASSAY

Typical Standard Curve



	gp120 pg/ml	A450 1	A450 2	A450 MEAN	A450 - Background	gp120 pg/ml Computed	A450 - Background Computed
STANDARD	2000.0	1.954	1.834	1.894	1.753	1997.0	1.755
HIV-1 gp120	1000.0	1.032	1.021	1.027	0.885	1007.1	0.879
	500.0	0.584	0.585	0.585	0.443	502.8	0.441
	250.0	0.350	0.354	0.352	0.211	237.5	0.221
	125.0	0.262	0.253	0.258	0.116	129.7	0.112
	62.5	0.194	0.205	0.200	0.058	63.5	0.057
DILUENT	0.0	0.153	0.140	0.142			
	0.0	0.136	0.137				

Regression Output:

Constant		0.002
Std Err of Y Est		0.007
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	