

HIV-1 p24 Core Profile ELISA

**Kit for the Detection and
Measurement of HIV-1 p24
Antigen/Antibody**

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HIV-1 p24 CORE PROFILE ELISA

INTENDED USE

This kit is designed to measure HIV-1 p24 core antigen (HIV-1 p24) and/or HIV-1 p24 core antibody binding capacity in serum, plasma, and cell culture media. This test is not intended to establish HIV seropositivity, but is restricted to use for monitoring patients.

SUMMARY AND EXPLANATION OF THE TEST

Acquired Immune Deficiency Syndrome (AIDS) is a disorder affecting cells of the immune system and is characterized by fatal opportunistic infections or neoplasms. AIDS and a variety of related disorders are associated with infection by a human retrovirus, known as Human Immunodeficiency Virus, type 1 (HIV-1). The DuPont HIV-1 p24 Core Profile ELISA is an enzyme immunoassay for the detection of HIV-1 p24 core antigen and core antibody binding capacity.

A 24 kilodalton protein (p24), immunologically distinct from proteins in most other retroviruses, has been demonstrated to be a major structural core component of HIV-1. The preparation of a mouse monoclonal antibody with high specificity and affinity for this viral protein has allowed the development of an extremely sensitive ELISA for HIV-1 p24 core antigen as well as a highly specific test for the measurement of HIV-1 anti-p24 binding capacity.

Several studies have shown that there are changing patterns of HIV-1 p24 antigen and antibody during the progression of clinical disease. Antigen is virtually undetectable and antibody is in excess during the early, asymptomatic stage. The onset and progression of disease is preceded by a marked decrease in antibody which is then followed by an increase in antigen (ibid). It has been suggested that a specific measurement of both HIV-1 p24 antigen and HIV-1 p24 antibody is of value. Adaptation of the Du Pont HIV-1 p24 antigen assay allows the researcher to also measure HIV-1 anti-p24 indirectly, but specifically, thus providing investigators with a versatile tool for HIV-1 research.

PRINCIPLE OF THE PROCEDURE

For detection of both HIV-1 p24 antigen and antibody, the DuPont HIV-1 p24 Core Profile ELISA is performed in two wells. The first well provides a measure of HIV-1 p24 antigen contained in the sample. A highly specific mouse monoclonal antibody to HIV-1 p24 core antigen immobilized to microplate wells, captures HIV-1 p24 core antigen that has been released upon lysis of virus in the sample. The captured antigen is complexed with biotinylated polyclonal antibodies to HIV-1 p24 core antigen and probed with a streptavidin-HRP (horseradish peroxidase) conjugate. The complex is detected by incubation with orthophenylenediamine-HCl (OPD) which produces a yellow color that is directly proportional to the amount of HIV-1 p24 core antigen captured. The absorbance of each well is determined using a microplate reader and calibrated against the absorbance of an HIV-1 p24 core antigen standard curve.

A separate well is designated for the measurement of HIV-1 anti-p24 relative binding capacity (RBC). A known concentration of HIV-1 p24 antigen (HIV-1 p24 spike) is added to the sample prior to assaying for HIV-1 p24 in the usual DuPont format. In the absence of endogenous antibody to HIV-1 p24 antigen in the sample, the HIV-1 p24 spike will be quantitatively recovered. However, in the presence of endogenous antibody, there will be a competition for this HIV-1 p24 spike between the antibody in the sample and the antibody coating the well. As a result, the quantity of HIV-1 p24 spike measured will decrease in relation to the amount of antibody present in the sample. If excess antibody is present in the sample, all of the HIV-1 p24 spike will be bound by sample antibody and none will be measured by ELISA. This decrease in HIV-1 p24 antigen recovery is used to calculate the RBC of a sample.

REAGENTS

Reagents are supplied for one, two or five 96-well microplate(s).

96-well microplate(s) coated with anti-HIV-1 p24.

plate covers.

HIV-1 p24 ELISA Viral Lysate Concentrate - 0.5 mL, 200 ng/mL as HIV-1 p24 (approx. 800 ng/mL as total HIV-1 protein), in Tris buffer containing detergent and 0.02% Sodium Azide as preservative. (For optional extended standard curve procedures.)

Positive Control - 5 mL, 0.4 ng/mL as HIV-1 p24 (approx. 2 ng/mL as total HIV-1 protein), in Phosphate Buffered Saline (PBS) containing added protein, detergent, and 0.1% Sodium Azide as preservative.

Negative Control - 13 mL recalcified human serum with detergent and 0.1% Sodium Azide as preservative. Non-reactive for HIV-1 p24 core antigen, Hepatitis B surface antigen, and antibody to HIV-1.

Standard/Sample Diluent - 123 mL PBS containing added protein, detergent, and 0.1% Sodium Azide as preservative.

Detector Antibody - 10 mL in PBS containing added proteins and 0.1% Sodium Azide as preservative. Contains human serum non-reactive for hepatitis B surface antigen and antibody to HIV-1.

Streptavidin-HRP Concentrate - 0.3 mL in Citrate buffer with added protein, detergent and preservative.

Streptavidin-HRP Diluent - 14 mL PBS containing added protein, detergent and preservative.

OPD Tablets - 5 tablets per strip.

Substrate Diluent - 60 mL Citrate buffer containing 0.03% Hydrogen Peroxide.

Stop Solution - 12 mL 4N Sulfuric Acid.

Triton X-100 - 7 mL, 5% in Phosphate buffer, with 0.02% Sodium Azide as preservative, and inert blue dye.

A 3.5 liter bottle of Plate Wash Buffer at 10X concentrate will be shipped with each HIV-1 p24 Core Profile ELISA System under separate cover. Additional ELISA Plate Wash Buffer (Cat. No. NEA-107) can be ordered from DuPont.

STORAGE RECOMMENDATIONS

Stop Solution and Plate Wash Concentrate may be stored at room temperature.
All other kit components should be kept refrigerated at 2° - 8°C.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

Changes in the physical appearance of the reagents supplied may indicate instability or deterioration of these materials. Do not use reagents which are visibly turbid.

OPD Substrate solution should be colorless or very pale yellow. A yellow-orange color indicates deterioration and the solution must NOT be used. The Substrate solution should be prepared within 30 minutes of use and protected from light.

WARNINGS AND PRECAUTIONS

1. Handle assay specimens, positive and negative controls, detector antibody, and HIV-1 viral lysate standards as if capable of transmitting an infectious agent.

The HIV-1 viral lysate standard and Positive Control supplied have been inactivated by psoralen, UV light and detergent treatment. However, because no known method can offer full assurance that infectious agents are absent or have been completely inactivated, these components must be handled using good laboratory practice to avoid skin contact and ingestion.

2. Do not pipet by mouth.
3. Wear disposable gloves throughout the test procedure. Dispose of gloves in the biohazard waste. Thoroughly wash hands afterwards.
4. Wipe spills promptly with 1% sodium hypochlorite (1:5 dilution of liquid household bleach). Contaminated materials should be disposed of in the biohazard waste.
5. Dispose of all materials and specimens used in the Du Pont HIV-1 p24 Core Profile ELISA in the biohazard waste. The recommended method of disposal is autoclaving for a minimum of 15 minutes at 121°C. Disposable materials may be incinerated. Mix liquid wastes with an equal volume of 5% sodium hypochlorite allowing at least 60 minutes for disinfection.
6. OPD is toxic, an irritant, a sensitizer, and is also classified as a potential carcinogen. Avoid inhalation and contact with the skin.
7. The Stop Solution contains 4 N Sulfuric Acid and is caustic. Avoid contact with the skin or mucous membranes.
8. Some of the reagents contain sodium azide as a preservative. If these materials, either concentrated or diluted, are to be disposed of through a sink or other common plumbing system, flush with generous amounts of water to prevent accumulation of potentially explosive materials.

EQUIPMENT REQUIRED BUT NOT PROVIDED

In addition to the reagents supplied with the kit, the following materials are required:

1. Suitable twelve channel or eight channel precision pipets with tips. Single channel repipettors may be used.
2. Vortex mixer.
3. Polypropylene tubes for preparation of standards and samples.

4. Disposable gloves.
5. Disposable reagent reservoirs.
6. Syringe-multichannel port manifold apparatus for manual plate wash dispensing OR automated plate washer.
7. Pump and vacuum dome or aspirator flask. If vacuum dome is unavailable, a double trap system is recommended.
8. Incubator capable of maintaining $37^{\circ} \pm 1^{\circ}\text{C}$.
9. Microtiter plate reader with 490 or 492 nm and > 600 nm filter capability. Follow installation, operation, calibration, and maintenance instructions provided by manufacturer.

PERFORMANCE CONSIDERATIONS

- Do not use kit components beyond the expiration date.
- Use only the reagent lots assigned to the kit. Do not interchange vials or bottle caps and stoppers.
- Equilibrate all reagents to room temperature (15° to 30°C) before use.
- Prepare all working reagents within 30 minutes of use. Prepare only enough for the assay being run. Discard any excess.
- After completion of each wash step, samples or the next reagent should be added promptly. **DO NOT ALLOW PLATE TO DRY AFTER WASHING.**
- Plate washing may be automated, semi-automated or manual, but **MUST BE CARRIED OUT WITH CARE** to ensure optimal performance of the assay. We recommend that at least ten remove-fill cycles be performed as below:
 - Automatic Microplate Washer - Use two 5-cycle washes of at least 300 L per well per wash. After each cycle wash, blot the plate by inverting and firmly tapping it on absorbent paper. Also, reorient the plate in the washer between cycles by turning it 180° .
 - Manual Microplate Washer - Wash ten (10) times, using 300 L per well per wash. Fill the entire plate, then aspirate in the same order. Blot the plate after the third and the last wash.
 - Hand-held Syringe or Squirt Bottle - Wash ten (10) times, using 300 L Diluted Plate Wash Concentrate per well per wash. Blot the plate after each wash.

- For HIV-1 p24 RBC assay, dilute all samples and prepare HIV-1 p24 spikes prior to pipetting samples into wells. Proceed without interruption to add all samples to wells.

SAMPLE PRETREATMENT

A. HIV-1 p24 Antigen Assay

a. Cell Culture Media:

Cell-free supernatants: In a small test tube, add 50 μL of the 5% Triton X-100 to 450 μL of cell-free media to make a final concentration of 0.5% Triton X-100. Mix.

Cell culture concentrate: Reconstitute and disrupt the concentrated pellet with 500 μL of the Standard/Sample Diluent.

NOTE: As different culture media are possible, we recommend that the user compensate for assay effects by replacing the appropriate portion of the Standard/Sample Diluent for making standards with the specific sample matrix to ensure that the reference curve matrix is as identical as possible to the sample matrix.

b. Serum/Plasma Samples

Pipet 50 μL of 5% Triton X-100 stock into a polypropylene test tube. Add 450 μL of serum or plasma to the test tube to make a final concentration of 0.5% Triton X-100. Clear, non-hemolyzed specimens should be used whenever possible. Mix well.

B. HIV-1 p24 Relative Binding Capacity Assay

a. Serum/Plasma Samples

Into a polypropylene test tube, pipet 10 μL of serum or plasma, followed by 990 μL of Standard/Sample Diluent provided with the kit. Pipet 100 μL of each diluted sample into another polypropylene test tube.

b. Diluted Negative Control

Into a polypropylene test tube, pipet 10 μL of Negative Control provided with the kit, followed by 990 μL of Standard/Sample Diluent. Pipet 100 μL of this diluted Negative Control into another polypropylene test tube.

c. Diluted Positive Control

Prepare a solution of HIV-1 p24 antigen at 0.125 ng/mL by diluting 2 mL of the Positive Control (0.4 ng/mL) with 4.4 mL of Standard/Sample Diluent. This dilution is enough for 15 samples in duplicate. If more samples are run, prepare additional diluted HIV-1 p24 antigen in the same ratio.

ASSAY PROCEDURE

Measurement of HIV-1 p24 Antigen and p24 Relative Binding Capacity (RBC) can be performed on the same plate. If only HIV-1 p24 Antigen measurement is desired, omit step 6 below.

1. Calculate the number of strips needed for the assay. Remove any excess strips from the holder and store in the Zip-Lock bag provided along with the desiccant packet. **CAUTION:** Whenever inverting the microplate to decant or blot, press side tabs of frame inward to prevent strips from falling out.
2. Dilute Plate Wash Concentrate (1:10) with distilled deionized water. Crystals that may form in the Plate Wash Concentrate if refrigerated should be redissolved by gentle warming prior to use. Unused diluted Plate Wash may be stored for up to 1 week at room temperature.
3. Prepare dilutions for a series of four standards from the Positive Control using the Negative Control provided with the kit. When running culture supernatants only, standards should be prepared using the Standard/Sample Diluent provided. Table I shows a suggested dilution scheme. If desired, serum or plasma which does not contain HIV-1 antigen or antibodies and which has an absorbance value within 25% of the Negative Control may be used to prepare the standards.

Table I

HIV-1 p24 Antigen Standard Curve Dilution Scheme

HIV-1 p24 Conc.	Tube	Diluent	+ Addition
100.0 pg/mL	A	750 μ L	250 μ L of Positive Control
50.0 pg/mL	B	500 μ L	500 μ L of A
25.0 pg/mL	C	500 μ L	500 μ L of B
12.5 pg/mL	D	500 μ L	500 μ L of C

4. Prewash the plate as described in Performance Considerations. Blot well.

IMPORTANT: To prevent plate from drying out, all samples must be added within 15 minutes. In the event that sample addition will exceed 15 minutes, a plate cover provided with the kit can be used effectively to seal pre-washed wells prior to sample addition.

5. HIV-1 p24 Antigen Assay - Sample and Standard Addition
 - a. Pipet 200 μ L of each pretreated sample into duplicate empty wells, G1,2; H1,2; etc. See Figure 1.
 - b. When assaying TISSUE CULTURE samples, pipet 200 μ L of Standard/Sample Diluent into wells A2 and B1,2. When assaying SERUM OR PLASMA samples, pipet 200 μ L of Negative Control Reagent into

wells A2 and B1,2. A1 will serve as the Substrate Blank and A2 and B1,2 as Negative Control wells.

- c. Pipet 200 μ L of each Standard A - D into duplicate wells F1,2 through C1,2.

6. HIV-1 p24 Relative Binding Capacity Assay - Sample and Spike Addition

NOTE: Refer to Sample Pretreatment, page 6.

- a. Pipet 400 μL of the diluted Positive Control (step B.3) into each tube containing the 100 μL serum/plasma (step B.1). Prepare the HIV-1 p24 control spike by pipetting 400 μL of the diluted Positive Control (step B.3) into the tube containing the 100 μL of diluted Negative Control (step B.2). Vortex all tubes briefly. Each serum/plasma sample is now at a final dilution of 1:500 and contains 0.1 ng/mL of HIV-1 p24.
- b. Pipet 200 μL of HIV-1 p24 control spike into duplicate empty wells. Each HIV-1 p24 control spike is now at a concentration of 0.1 ng/mL (100 pg/mL). See Figure 1.
- c. Pipet 200 μL of each spiked serum/plasma sample into duplicate empty wells.

IMPORTANT: Do NOT add the diluted Positive Control to the serum/plasma samples and HIV-1 p24 control spike until just prior to pipetting these samples into the assay wells.

Figure 1

Format for Microplate as Described in Assay Protocol

Key to Wells

SB = Substrate Blank

A-D = Standards

S = Samples for HIV-1
p24 Antigen Assay

NC = Negative Control

CS = HIV-1 p24 Control
Spike

RS = Samples for HIV-1
p24 RBC Assay

7. Cover plate and incubate two hours at $37^{\circ} \pm 1^{\circ}\text{C}$.

8. Wash plate as described. Blot well.
9. Add 100 μ L Detector Antibody to all wells except Substrate Blank, A1. Cover plate and incubate one hour at $37^{\circ} \pm 1^{\circ}\text{C}$.
10. Wash plate as described. Blot well.

- Dilute sufficient Streptavidin-HRP Concentrate 1:50 with Streptavidin-HRP Diluent. Protect from light before use. To determine amount of reagent required, see the Reagent Dilution Scheme in Table II. Mix thoroughly.

Table II

Streptavidin-HRP Dilution Scheme

# Strips Used	Streptavidin-HRP Concentrate	Streptavidin-HRP Diluent
4 strips	0.080 mL	4.0 mL
6 strips	0.120 mL	6.0 mL
8 strips	0.160 mL	8.0 mL
12 strips	0.240 mL	12.0 mL
24 strips	0.440 mL	22.0 mL

Add 100 L to all wells except Substrate Blank, A1. Cover plate and incubate 15 minutes at $37^{\circ} \pm 1^{\circ}\text{C}$.

- Wash plate as described. Blot well.
- Prepare sufficient OPD Substrate Solution within ten minutes of use as follows:

With non-metallic forceps or the equivalent, add one OPD Tablet to 11 mL of Substrate Diluent for each plate or partial plate assayed. Vortex vigorously to assure complete dissolution. Protect from light. The OPD solution should be colorless or pale yellow. A yellow-orange color indicates that the reagent is contaminated and must be discarded.

Add 100 μL to all wells and incubate 30 minutes at room temperature (18 to 24°C) in the dark.

- Stop the reaction by adding 100 μL of Stop Solution to all wells.
- Be sure bottom of plate is clean and dry. Read the plate at 490 or 492 nm, blanking the plate reader on air. Consult plate reader Instruction Manual for specific directions for instrument blanking. Readings must be taken with a reference filter at > 600 nm. When read against air, the Blank must have an absorbance reading of less than 0.050 for the assay to be valid.

CALCULATIONS OF CONTROLS AND SAMPLE VALUES

- Average the optical densities (O. D.) for each Standard and Sample run in duplicate to obtain the mean O. D.
- The Substrate Blank, when read against air, must have a reading of less than 0.050

for the assay to be valid. In addition, at least two of the three Negative Controls must have a reading of less than 0.150. If the assay is not valid, all specimens on that plate must be repeated.

3. HIV-1 p24 Antigen Assay

Calculate the Reactive Threshold Value (RTV) to determine if a sample is positive.

a. Tissue Culture Samples

Add 0.050 absorbance units to the mean absorbance unit of the Sample Diluent Negative Controls to obtain the Reactive Threshold Value.

$$\text{Example: } 0.020 + 0.050 = 0.070 = \text{RTV}$$

b. Serum and Plasma Samples

Add 0.080 absorbance units to the mean absorbance unit of the Negative Controls to obtain the Reactive Threshold Value.

$$\text{Example: } 0.020 + 0.080 = 0.100 = \text{RTV}$$

4. Compare the O. D. of each sample to the Reactive Threshold Value. Samples with O. D. values equal to or greater than the Reactive Threshold Value are considered reactive and should be repeated or confirmed.
5. Plot the mean O. D.'s for each Standard (y-axis) versus the concentration of HIV-1 p24 (x-axis) using graph paper or quadratic regression methods. See Table III and Figure 2.
6. Determine the concentration of HIV-1 p24 for each POSITIVE SAMPLE by interpolation from the standard curve.
7. Multiply the sample HIV-1 p24 concentration (pg/mL) by 1.11 to correct for the 0.5% Triton X-100 dilution. Final sample values should always be corrected for dilutions made during sample preparation.

NOTE: Reagents for the confirmation of positive samples can be ordered from DuPont (Cat. No. NEK-059, HIV-1 p24 Confirmatory Reagents).

8. HIV-1 p24 Relative Binding Capacity Assay

- a. Determine the concentration of recovered HIV-1 p24 antigen for the HIV-1 p24 control spike and for each sample by interpolation from the standard curve. The HIV-1 p24 control spike should have a concentration equal to or within 15% of 0.1 ng/mL.
- b. Subtract the concentration of recovered HIV-1 p24 antigen obtained for each serum/plasma sample from the concentration of recovered HIV-1 p24 antigen obtained in the HIV-1 p24 control spike. This result is "antibody-bound" HIV-1 p24.

- c. Multiply antibody-bound HIV-1 p24 (ng/mL) by the final dilution of sample (i.e. 500) to calculate the relative binding capacity (RBC) in ng/mL of the sample.

EXAMPLE:

1. Results Obtained: HIV-1 p24 control spike = 0.1 ng/mL
1:500 sample = 0.04 ng/mL
Calculations: RBC = (0.10 - 0.04 ng/mL) x 500 = 30 ng/mL

Figure 2

Table III

Typical Standard Curve

HIV-1 p24 Standard Concentration	Mean O.D. (490/620)
----------------------------------	------------------------

0 pg/mL	0.019
12.5 pg/mL	0.205
25 pg/mL	0.412
50 pg/mL	0.726
100 pg/mL	1.318

LIMITATIONS OF PROCEDURE

- A. The following materials have been checked and found to exhibit no detectable cross-reactivity:

Uninfected OKT4+ Cell Lines	H9	Molt3a	Molt4
Uninfected Monocyte Lines	U-937	MonoA 3.5	MonoA 4.5
Uninfected mixed PBL Cultures			
Azidothymidine	0.5 millimolar		
Dideoxycytidine	0.5 millimolar		
Ribavirin	0.5 millimolar		
HPA-23	0.5 millimolar		
Foscarnet	5 millimolar		

Similarly, no cross-reactivity was detected with culture fluid from 2 herpes simplex virus isolates, 2 cytomegalovirus isolates and 5 Epstein-Barr virus isolates.

- B. Reactivity was found in the following HIV isolates: 3B, RF, Z84, Z34, AL and MN.
- C. As noted in the Sample Pretreatment section, the researcher is responsible to test for differences in the specific sample matrix used in his/her assay and to appropriately compensate by replacement of a portion of the diluent for preparation of standards.

PERFORMANCE CHARACTERISTICS

- A. Reproducibility

Precision was determined by multiple duplicate analyses of HIV-1 viral lysate pools. Typical results are given below in pg/mL.

Within Assay Variation					Between Assay Variation				
Sample	n	Mean	SD	CV	Sample	n	Mean	SD	CV
A	12	49.7	2.8	5.5%	A	12	53.9	4.0	7.5%
B	12	94.3	2.5	2.7%	B	12	100.7	6.2	6.2%

- B. Linearity

The effect of some typical matrices for the assay were evaluated by spiking to a level of 200 pg/mL with HIV-1 viral lysate followed by assay of the original and serial dilutions made in assay buffer. Values were then corrected for dilution.

MATRIX	NORMALIZED VALUES (pg.mL)			
	Undiluted	1:2	1:4	1:8
Std/Sample Diluent (Kit)	> 100	187	200	196
Normal Human Plasma	> 100	187	200	206

Dilution of different matrices such as "RPMI", cell-free supernatants, and disruption buffer were also linear throughout the range of the assay. Dilution of these matrices with assay buffer had no significant effect on the normalized value.

C. Sensitivity

The sensitivity of this kit was determined to be 2.5 pg/mL of HIV-1 p24 antigen for culture supernatants and 4.4 pg/mL for serum and plasma samples. The cut-off algorithm has been statistically derived from a study of normal donor samples to allow assay sensitivity to the lowest concentration in the standard curve and to maximize specificity.

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