HIV-4th GEN. Ag & Ab COMBO ELISA TEST KIT

PAREEKSHAK®

For professional use

A Solid-Phase enzyme immunoassay for the simultaneous detection of HIV Antigen and Antibodies in Human Serum or Plasma

IVD

READ THE PACK INSERT BEFORE USE PROVIDED ALONG WITH THE KIT

REF : HIV-Combo

HIV- 4th GEN. Ag & Ab COMBO ELISA TEST KIT is an In vitro Antigen and Antibody sandwich elisa where in the HIV antigen p24 and Isotypes of HIV antibodies are detected simultaneously along with antibodies to HIV-2.This method enables the early detection of HIV reducing the window period of detection.

INTRODUCTION :

HIV-4th GEN. Ag & Ab COMBO ELISA TEST is an immunoassay which employs r-proteins of HIV-1 gp41, C-terminus of gp120, HIV-2 recombinant protein of gp36 along with Monoclonal Anti p24 antibodies to HIV-1 for the detection of antigen & antibodies to HIV 1&2 in human serum or plasma. These proteins and antibodies, which are corresponding to highly antigenic segments of both the structural and non-structural proteins of the HIV constitute the solid phase antigenic absorbent. The use of r-proteins offers the advantage of high degree of specificity and sensitivity due to multiple epitopes. Parallel addition of monoclonal Anti-p24 antibodies will enhance the sensitivity of the detection by reducing the window period of HIV detection. This enables elimination of great risk of contracting the HIV through blood transfusions which is known to have serious limits on detection of early sero conversion samples. The epidemiological evidence indicates that an infectious agent transmitted through intimate contact, intravenous drug use or use of infected blood or blood products, leads to Acquired Immunodeficiency Syndrome (AIDS).This infection affects T-Cell mediated immunity, resulting in severe lymphopenia and a reduced sub-population of helper T lymphocytes. Destruction of this T-lymphocyte population by the virus cause an irreversible damage to immune system, resulting in a reduced or deficient response to subsequent infections and hence the patient becomes vulnerable (prone) to all kind of infections and shows bizzare symptoms hence this condition called as Syndrome. Consequently, infections become more severe and may cause death. At present there is no successful treatment for AIDS. The etiological agent has been identified as a retrovirus, human immunodeficiency virus type 1 (HIV-1). A closely related, but distinct second type of immunodeficiency virus, designated as HIV-2, has been isolated and causes a disease that is indistinguishable from AIDS. The only difference is in the potentiality of infection. Serological cross reactivity between HIV-1 and HIV-2 has been shown to be highly variable from sample to sample. This variability necessitates the inclusion of antigens to both HIV-1 and HIV-2 for the detection of HIV-1 and HIV-2. The HIV genome has outer structural (env-gp120, gp41), inner structural (gag p17, p24, p7, p6), pol-viral enzymes (protease, reverse transcriptase, integrase) and regulatory proteins (Tat, Rev, Vif, Vpu, Vpr, Nef) and long terminal repeats on either end (Fig. 1).

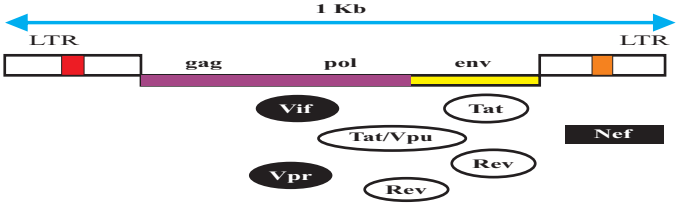


Fig. 1 **Structure of HIV genome**

Index:

I. gag = P17 | P24 | P 7 | P 6 (Inner structural proteins of the retro virion)

II. Pol = PR | RT | IN

(encodes the viral Enzymes: PR - protease, RT = Reverse Transcriptase, IN =integrase)

III. Env = gp 120 | gp 41 (Outer envelop glycoproteins - associated with lipid bilayer)

IV.It also Encodes For 6 small proteins unique to the virus. Tat & Rev - positive Regulatory protein Vif. Vpu Vpr. Nef - proteins with accessory function LTR -Long terminal repeat at each end. The left or 5'LTR containing the signals for transcription initiation & the right or 3' LTR contains the signals for transcription termination.

HIV- 4th GEN. Ag & Ab COMBO ELISA TEST KIT utilizes a unique combination of HIV-1 & 2 antigens of the virus to selectively detect all subtypes of HIV-1 & 2 Virus in human serum/plasma with a high degree of sensitivity and specificity along with simultaneous detection of HIV p24 antigens in human serum or plasma.The level of different type of antibodies and antigens of HIV in blood is as shown in Fig. 2

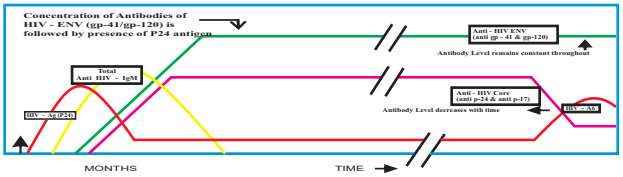
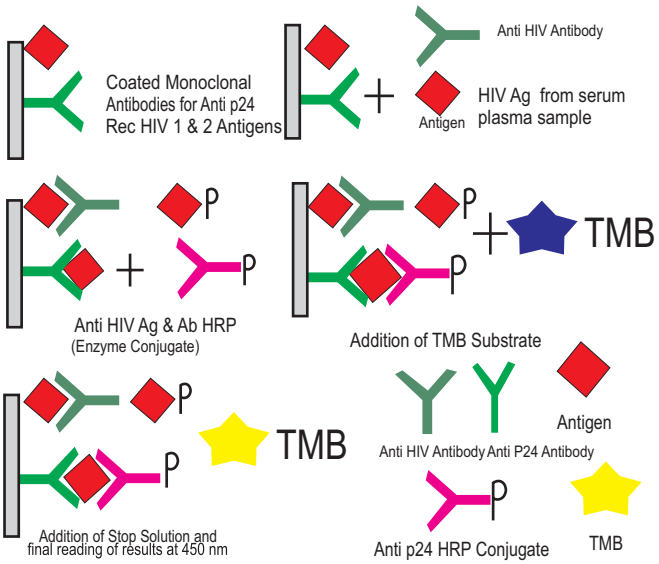


Fig. 2 **Level of different type of antibodies and antigens of HIV in blood**

TEST PRINCIPLE:

HIV- 4th GEN. Ag & Ab COMBO ELISA TEST KIT utilizes a unique combination of HIV-1 & 2 antigens of the virus to selectively detect all subtypes of HIV-1 & 2 Virus in human serum/plasma with a high degree of sensitivity and specificity along with simultaneous detection of HIV P24 antigens in human serum or plasma.

Schematic Representation of "HIV 4th gen Ag & Ab Elisa Kit"



STORAGE AND STABILITY :

Store the kit between 2-8°C. DO NOT FREEZE. The bag containing microtiter plate must be brought to Room temperature (20-30° C) before opening. To avoid condensation in the wells unused wells should be sealed in the bag and refrigerated (2-8° C). After opening the sealed pouch, unused strips are stable for 3 months at 2-8°C in the original pack sealed with tape. Do not return the holder to the pack.

STABILITY:

1.The unopened kit is stable for 18 months from the date of manufacturing as indicated on the package when stored in recommended storage conditions.

2.The opened kit is stable for 3 months from the date of opening.

3.Repeated freeze thaw of reagents from 2-8°C to Room temperature several times will reduce the stability of the kit.

PACK SIZE : Available in packs of 48 Tests,96Tests & 480 Tests.

CONTENTS OF THE KITS :

Materials	48 Test	96 Test	480 Test
Anti-P24 & HIV Rec Antigen coated microwells (Ready to use)	8 wellsX6 strips	8 wellSX12 strips	96 wellsX 5 Plates
Wash solution (concentrated 10x)	50ml	100ml	5x100ml
Biotinated Anti-P24 Antibody Conjugate (50x Concentrated)	0.075ml	0.150ml	5x0.150ml
Enzyme Conjugate Dilluent	10ml	20ml	5x20ml
Streptavidin-HIV-1/2 HRP Conjugate (100x Concentrated)	0.075ml	0.150 ml	5x0.150ml
TMB Substrate	4ml	8ml	5x8ml
TMB Diluent	4ml	8ml	5x8ml
Stop Solution (Ready to use)	6ml	12ml	5x12ml
Positive Control (Ready to use)	0.5ml	1ml	5x1ml
Negative Control (Ready to use)	0.5ml	1ml	5x1ml
Adhesive Slips	2 Nos	3 Nos.	15 Nos.
Pack Insert	1 No.	1 No.	1 No.

PRECAUTIONS:

1. For in vitro diagnostic use only.
2. The positive control contains inactivated HIV Antibodies and Cultured HIV p24 antigen. However, it should be treated as infectious. The Negative serum also should be treated as infectious.
3. All human serum and plasma samples should be considered potentially infectious. It is recommended that all specimens of human origin should be handled as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease control/National Institute of Health Manual "Bio-safety in Microbiological and Biomedical Laboratories".1984.
4. Never pipette by mouth.
5. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
6. Wear disposable latex gloves while handling specimens and kit reagents. Afterwards wash hands carefully with disinfectants. Avoid splashing or forming aerosols.
7. Discard all materials and specimens capable of transmitting infection. The preferred method of disposal is autoclaving for a minimum of one hour at 121°C.
8.Liquid wastes not containing acid may be mixed with sodium hypochlorite in volumes such that the final mixture contains 50-500mg/dl available chlorine. Allow 30 minutes for decontamination to be completed.

NOTE :

1. Liquid wastes containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite.
2. Spills should be wiped up thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Materials used to wipe up spills should be added to bio hazardous waste matter for proper disposal.
3. Deterioration is indicated by a significant decrease in the absorbance level of positive control.
4. Avoid exposure of TMB solution to intense source of light. Oxidising agents, metallic ions or soap remaining in glassware containers can interfere with the TMB reaction. In order to avoid this problem rinse the glassware thoroughly with 1N acid (HCl or H_2SO_4) followed by several washes with distilled water before use.
5. Reagents should be stored between 2-8° C. Avoid unnecessary exposure to light. This is merely a precaution. The light sensitive reagents are the conjugate and the TMB. Storage of reagents and samples in self defrosting freezers is not recommended.
6.Do not use reagents after expiration date mentioned on the label.
7. Do not mix or interchange reagents from different kit or kit lots. Cross contamination of reagents or samples can cause erroneous results.
8. Stop solution contains sulphuric acid. Avoid contact with skin & eyes
9. Do not interchange vial caps.
10. When removing aliquots from the reagent vials, use aseptic technique to avoid contamination, otherwise incorrect results may occur. Use a new pipette tip for each sample. Optimal results will be obtained by strict adherence to the protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements are essential.
11.Once the assay has been started, all steps should be performed without interruption.
12.Do not touch the wells or scratch the wells while pipetting.
13.Do not let wells dry once the assay has started.
14. Reusable glassware's must be disinfected, washed out and rinsed free of detergents.
15. Use separate tips for TMB SUBSTRATE and TMB DILUENT

INDICATIONS OF INSTABILITY AND DETERIORATION OF REAGENTS

1. Changes in the physical appearance of the reagents supplied may indicate deterioration of these materials. Do not use reagents, which are visibly turbid.
2. The TMB SUBSTRATE solution should be colorless for proper performance of the assay. Any color may indicate deterioration of the TMB substrate.

PREPARATION OF REAGENTS:

Wash buffer preparation:

1. Dilute the wash solution 1/10 with distilled or de-ionised water. Diluted wash solution should be stored at 2-8°C and is stable for 2 weeks. If the concentrated solution shows any crystals, dissolve them by warming in a water bath at 37°C before dilution.for eg.: mix 1ml of wash solution and 9ml of distilled water

2.Preparation of Working Biotinated Anti-p24 Antibody conjugate (BEFORE USE ONLY):
Mix HIV-4th GEN. Ag & Ab COMBO ELISA TEST KIT Biotinated Anti-p24 Antibody conjugate Concentrate and Diluent 1:50 ratio to prepare working Antibody conjugate for eg :
For 8 Wells Mix 0.5 ml of Enzyme conjugate Diluent and 10 µl of Concentrate Biotinated Anti-P24 Antibody Conjugate.

3. Preparation of Streptavidin HIV1/2 HRP Conjugate (Before use only)
Mix HIV-4th GEN. Ag & Ab COMBO ELISA TEST KIT Streptavidin HIV 1/2HRP Conjugate Concentrate and Enzyme Diluent 1:100 ratio to prepare working Conjugate. fr eg :for 8 wells Mix 1 ml of Enzyme Diluent and 10µl of Concentrate Enzyme Conjugate.

4.Preparation of Working substrate (BEFORE USE ONLY):
Mix TMB Substrate and TMB Diluent in 1:1 ratio to prepare working Substrate. for eg :
For 8 Wells Mix 0.5 ml of TMB Substrate and 0.5 ml of TMB Diluent.
NOTE : Prepare working Substrate solution freshly every time mix solution thoroughly before use.

TEST PROCEDURE:

1. Wear disposable latex gloves throughout the procedure.
2. Bring all reagents and Micro wells to Room temperature (25-30°C) before starting the assay. Gently mix all liquid reagents before use.
3.Dilute the wash solution 1/10 with distilled or de ionized water.
4.Set up micro titer wells in the frame provided.
5.Label A1 as Blank, B1 & C1 as Negative control and D1, E1 and F1 as Positive controls.
6.Add 50µl of control or test sample to appropriately labeled wells of the micro titer plate.
7.Add 50 µl of Diluted Biotinated Anti P24 Antibody conjugate solution to each well Except Blank and mix thoroughly by gentle swirling.
8.Cover the wells with adhesive slips.
9.Incubate at 37°C for 60 minutes.
10.Wash the micro plate 5 times by adding 300µl (approximately) of with working wash solution Each well.
11. Add 100 µl of Diluted Streptavidin-HIV 1/2HRP conjugate solution to each well Except Black and mix thoroughly by gentle swirling.Incubate at RT for 30 minutes
12. Wash the micro plate 5 times by adding 300µl (approximately) of each well with working wash solution.
13. Add 100µl of working substrate solution including blank.
14.Incubate at RT for 30 minutes (25°C to 30°C) Avoid expose to light
15. Add 100µl of stop solution to each well including blank.
16. Read the absorbance at 450nm on an ELISA Reader within 30 minutes. Use of reference filter 620-630 nm is advisable.

RESULTS QUALITY CONTROL VALUES

Blank value should be lesser than 0.15
Test validity: **NEGATIVE CONTROL MEAN (NCx):** Individual negative control values should be less than or equal to 0.250 when the photometer is blanked against reagent blank. If one of the values is outside the acceptable range, discard this value and recalculate the mean. If two of the values are out of range, the test should be repeated.
POSITIVE CONTROL MEAN (PCx) : PC value should be more than 0.6
To achieve the expected detection limit the value of PCx minus Ncx should be greater than or equal to 0.6. If not, the technique may be suspected and the assay should be repeated.

CALCULATION OF THE MEAN CONTROL VALUES

Example

Negative control Sample No.	Absorbance	Positive control Sample No.	Absorbance
1	0.038	1	2.523
2	0.030	2	2.505
		3	2.490
Total	0.068	Total	7.518

Ncx = $\frac{\text{Total absorbance}}{2} = \frac{0.068}{2} = 0.034$ Pcx = $\frac{\text{Total absorbance}}{3} = \frac{7.518}{3} = 2.506$

CALCULATION OF THE CUT-OFF VALUE (COV)

Determine the cut-off value by adding 0.1 to the negative control mean (Ncx). This cut-off value is used to achieve the highest possible sensitivity eg.

CUT OFF FORMULA = NCx + 0.1

Example : COV = 0.034+0.1 = 0.134

RESULTS :

1. Non-Reactive :
A test sample is considered to be non-reactive for HIV Ag & Ab if the resulting absorbance value is less than the cut-off value.

2. Reactive :A test sample is considered to be reactive for HIV Ag & Ab if the resulting absorbance value is greater than or equal to the cut-off value.

INTERPRETATION OF RESULTS :

1. Specimens with absorbance values less than the cut-off value are considered non-reactive by HIV-4th GEN. Ag & Ab COMBO ELISA may be considered negative for HIV Antigen and Antibody. Further testing is not required when correlated clinically. When the clinical correlation is not satisfying the results sample should be investigated for confirmatory tests such as PCR methods and ECLIA methods as per the guidelines of local authorities respectively.
2. If the values are 10% less or more than the cutoff value (Border line), then the samples must be retested.
3. The OD values on 450/630nm filter can come in negative (-) values which in fact does not have any effect on the results and instead shows the great extent of specificity.
4. Specimens with absorbance value greater than or equal to the cutoff value are considered initially reactive by HIV-4th GEN. Ag & Ab COMBO ElisaTest. The original sample should be retested in duplicate, before final confirmation

a)Initially reactive specimens which do not react in either of the duplicate, repeat tests are considered negative for HIV 4th Gen Ag & Ab Elisa Test. Further testing is not required.
b)Initially reactive specimens which are reactive in one or both of the repeat tests are considered repeatable reactive.
c)As in any diagnostic enzyme immunoassay, there is a possibility that repeatable reactions may occur for the following reasons. A test sample is considered to be reactive for HIV Ag & Ab if the resulting absorbance value is greater than or equal to the cut-off value.
Inadequate washing Contamination of reaction well with HRP conjugate Contamination of substrate solution with conjugate or with oxidizing agents. Cross-contamination of non-reactive specimens by HIV Ag & Ab.

TROUBLE SHOOTING :

BLANK HAS TOO HIGH ABSORBANCE VALUES

Cause/Error	Remedy
1. Substrate solution is contaminated	Use fresh pipette tips every time
2. Contamination, spills from other wells	Avoid contamination
3. Washing solution has not been diluted correctly	Should be diluted 1/10 (1+9)
4. Poor washing	Check your washer

POSITIVE CONTROL HAS TOO HIGH ABSORBANCE VALUE

Cause/Error	Remedy
1. Substrate solution is contaminated	Use fresh pipette tips every time
2. Interchange of controls from different lots	Do not mix or interchange reagents from different lots.
3. The pipetted volume is too high	Volume should be 50µl

POSITIVE CONTROL HAS TOO LOW ABSORBANCE VALUES

Cause/Error	Remedy
1. Interchange of controls from different lots	Do not mix or interchange reagents from different lots.
2. The pipetted volume is too high	Volume should be as indicated

NEGATIVE CONTROL HAS TOO HIGH ABSORBANCE VALUES

Cause/Error	Remedy
1. Contamination, spills from other wells.	Avoid contamination or interchange of the vial caps.

ALL ABSORBANCE VALUES VERY HIGH

Cause/Error	Remedy
1. Interchange of reagents from different lots.	Do not mix or interchage reagents from different lots.
2. Substrate solution is contaminated	Use clean containers
3. Washing solution concentrate has not been diluted correctly.	Should be diluted 1/10 (1+9)
4. Poor washing	Check your washer
5. Contaminated solution containers	Use clean containers
6. Deterioration of reagents	Use aseptic technique. Do not pour used reagent back to vials.
1. Reagent solutions used after they have expired.	Do not use reagents after the expiration date
2. The reagents have not been warmed up to room temperature	Should be 25°-30°C when starting the assay
3. Once opened microtiter foil package has not been resealed tightly and stored properly with dessicant.	Once opened microtiter plate foil package has to be resealed tightly and stored properly with dessicant.
4. Interchange of reagents from different lots	Do not mix or interchange reagents from different lots
5. Substrate solution is exposed to direct sunlight.	Avoid unnecessary exposure to light
6. Stop solution has not been mixed properly before measurement	Mix the plate before measuring
7. Deterioration of reagents	Use aseptic technique. Do not pour used reagent back to vials.
8. Contamination of conjugate by human serum or plasma (usually from samples)	Even one microliter of human serum or plasma is enough to inhibit as much as 1 litre of conjugate
9. TMB chromogen have been too cold	Bring the TMB Chromogen to Room temperature

POOR SPECIFICITY

Cause/Error	Remedy
1. Washing solution has not been diluted correctly.	Should be 1:10 (1+9)
2. Salt crystals in the washing solution concentrate have not being redissolved before diluting.	Redissolve the crystals before diluting by warming and mixing the concentrate
3. Poor washing	Check your washer
4. Too low positive control value	See positive control has too low absorbance value

PERFORMANCE CHARACTERISTICS

ACCURACY

HIV- 4th GEN. Ag & Ab COMBO ELISA TEST meets the requirement for the fourth generation test when tested against the commercially available kits.HIV 4th Gen Ag & Ab Elisa was tested with the sero converison panels and also p24 standards quantified had resulted in 200 pg/ml sensitivity. However certain hyper immune status and infectious deseases known to cross react with immunoassays can interfere with the tests resulting false positives which need to be confirmed with other sensitive assays such as PCR and eCLIA assays but not with the Antibody test kits.

A. Precision Intra-assay

The intra-assay variation of HIV-4th GEN. Ag & Ab COMBO ELISA TEST KIT Was determined by testing positive and negative samples. Operator to-Operator variation was calculated from the results of Intra-assay variation study performed by three technicians. Summary of the results is as follows Table : Summary of the Intra-assay variation and Operator-to Operator variation study of HIV-4th GEN. Ag & Ab COMBOELISA TEST

Serum sample	Mean (O.D)(A450nm)	Standard Deviation (SD)	Coefficient of Variation (%)
NC	0.100	0.002	2.00
PC	2.678	0.037	1.38

B. Sensitivity :

No. of Positive samples tested	No. of Positives by HIV - 4th GEN. Ag & Ab COMBO ELISA Test	Sensitivity (%)
99	99	100%

No. of Negative Samples tested	No. of Negatives by HIV- 4th GEN. Ag & Ab COMBO ELISA Test	Specificity (%)
200	199	99.5 %

Samples Dilution	Result OD 450nm
Nil	2.533
1:1000	2.533
1:5000	2.624
1:10,000	0.881
1:20,000	0.349
1:40,000	0.257

Panel Member	Commercial HIV 4th gen Assays	HIV 4th Gen Ag & Ab Elisa
A	-	-
B	+	+
C	+	+
D	+	+

NOTE::

Even after the best effort is made to supply the product as per the sample submitted but due to continuous R & D, the company reserves the right to improve/change any specifications/components without prior information/notice to the buyer

LIMITED EXPRESSED WARRANTY OF MANUFACTURER

The manufacturer limits the warranty to this test kit, as much as that the test kit will function as an in vitro diagnostic assay within the Nature of Sample, Procedure limitations and specifications as described in the product instruction manual, when used strictly in accordance with the instructions contained. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

REFERENCES :

1.Samgadharan MG, Markham PD: The role of human T-lymphotropic retroviruses in leukemia and AIDS, in Wormser GP (ed): AIDS and Other Manifestations of HIV Infection. New Jersey, Noyes Publications, 1987, pp 218-220 2.Barre-Sinoussi F, Chermann JC, Rey F, et at: Science 220:868-871, 1983.

3.Gallo RC, Salahuddin SZ, Popovic M, et at: Science 224:500-503, 1984.

4.Coffin J, Haase A, Levy JA, et at: What to call the AIDS virus? Nature 321:10, 1986.

5.Clavel F, Guetard D, Brun-Vezinet F: Science 233:343-346, 1986.

BS ISO-15223-1:2012(E) MEDICAL DEVICES SYMBOL

2C 8C	Temperature Limitation	2001-06	Date of Manufacture	IVD	In vitro Diagnostic Device
LOT	Batch Code		Company name & address		Consult Instructions For Use
	Use by	Company	Company Name	EC REP	Authorised Representative in European Community
	Do Not Re use	Σ	Sufficient for		KEEP AWAY FROM SUNLIGHT
	KEEP DRY	NON STERILE	NON-STERILE	CONTROL −	NEGATIVE CONTROL
CONTROL +	POSITIVE CONTROL				

QUICK PROCEDURAL REFERENCE

Addition of Samples	50 µl
Prepare Working Enzyme (1)	Strips No 1 2 3 4 5 6 7 8 9 10 11 12 Enzyme Concentrate (µl) 10 20 30 40 50 60 70 80 90 100 110 120 Enzyme Diluent (ml.) 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0
Add Working Biotinated Anti P24 Ab Conjugate	50 µl
Cover the plate & incubate	60 min at 37 degrees
Wash	5 Cycles
Prepare Working Enzyme (2)	Strips No 1 2 3 4 5 6 7 8 9 10 11 12 Enzyme Concentrate (µl) 10 20 30 40 50 60 70 80 90 100 110 120 Enzyme Diluent (ml.) 1 2 3 4 5 6 7 8 9 10 11 12
Add working Sreptavidin HIV 1/2 HRP Conjugate	100µl
Cover the plate &Incubate	30 min at RT (25-30 degrees).
Wash	5 Cycles
Prepare TMB Substrate	Strips No 1 2 3 4 5 6 7 8 9 10 11 12 TMB Substrate (ml) 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 TMB Diluent (ml.) 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0
Add Substrate	100 µl
Cover the plate & Incubate in dark	30 minutes at Room Temp at (25-30 degrees).
Add Stop Solution	100 µl
Read Results	450 nm./630 nm.

SUMMARY OF PROCEDURE :

Add 50µl of Test sample or Controls

↓

Add 50 µl of Working Biotinated Anti-P24 Antibody Conjugate(1)

↓

Incubate 60 minutes at 37°C

↓

Aspirate and wash 5 times with working wash solution

↓

Add 100 µl of Working Sreptavidin HIV 1/2 HRP Conjugate(2)

↓

Incubate for 30 minutes at R.T. at (25-30 degrees)

↓

Aspirate and wash 5 times with working wash solution

↓

Add 100µl of Working Substrate solution

↓

Incubate for 30 minutes at R.T. at (25-30 degrees)

↓

Add 100µl of stop solution

↓

Read the absorbance at 450/630nm filter

Quick calculative information:

Blk, 2 NC, 3 PC
Validation:
Blank less than 0.15
Ncx Less than 0.25
Pcx above 0.6
Cut off Formula: NCx + 0.1
Filters: 450nm/620-630 nm

R-0,05/2014

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