HIV-1/2 ELISA KITS

PAREEKSHAK®

For Professional Use

An ELISA for the detection of antibodies to HIV-1 and HIV-2 in Human Serum / Plasma

HIV-1 Antigens for gp 120, gp41 HIV-2 Antigen for gp36

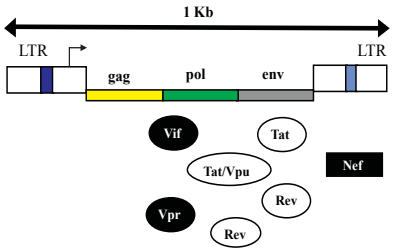
IVD

Read the pack Insert before use provided along with the kit

REF PEL

INTENDED USE : The Pareekshak® HIV ½ ELISA kit is a solid phase immunoassay for the detection of antibodies to HIV-1 and HIV-2 in human serum or plasma.

INTRODUCTION : 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 disease affects T-Cell mediated immunity, resulting in severe lymphopenia and a reduced subpopulation of helper T-lymphocytes.Destruction of this T-lymphocyte population by the virus causes an immune deficiency, resulting in a reduced or deficient response to subsequent infections.Consequently, infections become more severe and may cause death.



Index: **Fig. 1 Structure of HIV genome.**

I. gag =	p17	p24	p7	p6	
II. Pol =	PR	RT	IN		(encodes the viral enzymes : PR=Protease, RT=Reverse Transcriptase, IN=Integrace)
III. Env=	gp 120	gp 41			(outer envelop glycoproteins-associated with lipid

IV. Encodes also 6 small proteins unique to the virus. Tat & Rev –positive Regulatory protein Vif. Vpu. Vpr. Nef- proteins with accessory function.
V. LTR – Long terminal repeat at each end. The left or 5LTR containing the signals for transcription initiation & the right or 3' LTR contains the signals for transcription termination.
The Pareekshak HIV-1/2 ELISA utilizes a unique combination of HIV-1/2 antigens of the virus to selectively detect all types of HIV-1/2virus in human serum or plasma with a high degree of sensitivity and specificity. Test for the antibodies to HIV was proved to be highly valuable in diagnosis and study of the infection. The antibodies produced against HIV proteins are found in infected people and are useful tools for the diagnosis of HIV in blood is as shown in Fig.2

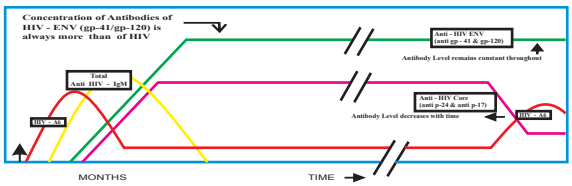
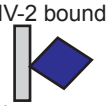


Fig. 2 **Level of different type of antibodies and antigens of HIV in blood**

PRINCIPLE :

1. Pareekshak® HIV1/2 ELISA employs an immunosorbent enzyme assay, which consists of recombinant protein for gp120, gp41 of HIV- 1, gp36 of HIV-2 bound to wells of the micro plate.

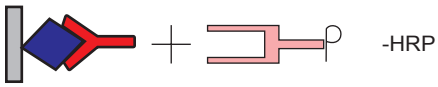


Antigen bound to microwell

2. During the course of the assay, diluted controls and diluted specimens are added to the wells and incubated. HIV specific antibodies, if present, will bind to the antigens.

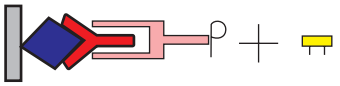
Antibody

3. After a thorough washing of the wells to remove unbound antibodies and other serum components, standardized preparation of horseradish peroxidase-conjugate is added to each well. This conjugate preparation is then allowed to react with antibodies which bind to the assay wells on the basis of their specificity for antigenic determinants present within the HIV antigens.



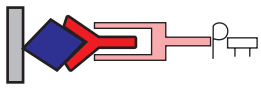
-HRP

4. After another thorough washing of the wells to remove unbound horseradish peroxidase-conjugated antibodies, a substrate solution containing hydrogen peroxide and TMB is added to each well. A blue colour develops in proportion to the amount of HIV specific antibodies present, if any, in the serum or plasma samples tested.



TMB Substrate

5. This enzyme-substrate reaction is terminated by the addition of a solution of sulfuric acid (H_2SO_4). The color changes to yellow that have occurred in each well are then measured spectrophotometrically at a wavelength of 450nm/630nm.



Blue color complex turns yellow on adding Stop Solution

STORAGE AND STABILITY :

STORAGE :

Store the kit between 2-8°C. DO NOT FREEZE. The bag containing micro titer 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.
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, 96 Tests & 480 Tests.

CONTENTS OF THE KITS :

Materials	48 Tests	96 Tests	480 Tests
Microtiter Plate (Ready to use)	6x8 wells strip	12x8 wells strip	96w x 5 plates
Dilution buffer (Ready to use)	7 ml	15 ml	5x15ml
Wash solution (Concentrated 10X)	50 ml	100 ml	5x100 ml
HRP Conjugate (Concentrated 100X)	0.2 ml	0.3 ml	5x0.3 ml
HRP Conjugate diluent	8 ml	15 ml	5x15 ml
TMB Substrate	4 ml	8 ml	5x8 ml
TMB Diluent	4 ml	8 ml	5x8 ml
Stop solution (Ready to use)	6 ml	12 ml	5x12 ml
Positive control (Ready to use)	0.5 ml	1 ml	5x1 ml
Negative control (Ready to use)	0.5 ml	1 ml	5x1 ml
Adhesive slips	2 No.	3 Nos.	5x3 Nos.
Pack Insert	1	1	1

MATERIALS REQUIRED BUT NOT PROVIDED :

1. Distilled or De-ionised water, preferably sterile
2. Graduated cylinders for reagent dilutions.
3. Vials to store the diluted reagents.
4. Precision pipettes.
5. Paper towels or absorbent paper.
6. Timer.
7. ELISA Reader.
8. ELISA Washer.
9. Sodium hypochlorite solution (free available chlorine 50- 500mg/dl)

SPECIMEN COLLECTION AND HANDLING :

Specimens must be centrifuged before use (e.g. 3000 RPM, 10 minutes), especially citrate plasma specimens will cause false HIV reactive results, if not centrifuged properly.
Serum and plasma (preferably EDTA) samples may be stored for up to 7 days at 2-8°C or atleast 6 months as frozen (-20°C or –70°C). Samples should not be repeatedly frozen and thawed.
Do not use heat-inactivated samples. Especially heat inactivated plasma specimens will cause false HIV reactive results.
Do not use sodium azide as preservative because it inactivates horseradish peroxidase. Microbial- contaminated, grossly hemolysed, icteric or lipemic serum and plasma specimens may give erroneous results.

PRECAUTIONS :

1. For in vitro diagnostic use only.
2. The positive control contains inactivated Hepatitis B virus, 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 “Biosafety 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. Wear disposable latex gloves while handling specimens and kit reagents. Afterwards wash hands carefully with disinfectants. Avoid splashing or forming aerosols. Discard all materials and specimens capable of transmitting infection. The preferred method of disposal is autoclaving for a minimum of one o hour at 121°C. 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 biohazardous 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 are 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 and 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.
10. Once the assay has been started, all steps should be performed without interruption.
11. Do not touch the wells or scratch the wells while pipetting.
12. Do not let wells dry, once the assay has started.
13. Use Separate Tips for TMB SUBSTRATE and TMB DILUENT
14. Reusable glassware's must be disinfected, washed out and rinsed free of detergents.

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.

TEST PROCEDURE & PRELIMINARY PREPARATIONS:

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-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 shaking and mixing or warming in a water bath at 37°C before dilution.
4. Preparation of working HRP conjugate: Dilute 100X HRP conjugate diluent. For eg. For 8 wells mix 10µl of 100X HRP conjugate and 1 ml of HRP conjugate diluent.
5. Preparation of working Substrate solution : 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 every time. mix solution thoroughly before use.

TEST PROCEDURE :

1. Set up the micro titration wells in the frame provided and label each well. Label one well as reagent blank (A1) and two wells each as Negative controls (B1 & C1) and Positive controls as (D1, E1 & F1).
2. In duplicate, add 100 µl of **READY TO USE** Positive (3 Nos) and Negative controls (2 Nos) to appropriately labeled wells of the microtiter plate.
3. Add 100 µl of Ready to use dilution buffer into required number of wells other than controls.
4. Add 10 µl of samples into each well and mix thoroughly by gentle swirling.
5. Cover the wells with adhesive slips.
6. Incubate at RT for 30 minutes (25-30°C).
7. Aspirate and dispose the samples along with microtips into a container containing 0.5 % sodium hypochlorite (ordinary bleach).
8. Wash the microplate 5 times with approximately 300 µl per well of working wash solution. Care should be taken to avoid overfilling and cross contamination.
9. Add 100 µl of the working HRP conjugate solution in the same order.
10. Incubate at R.T for 30 minutes (25-30°C).
11. Wash the micro plate 5 times with approximately 300 µl per well of working wash solution.
12. Add 100 µl of working TMB substrate to each well in the same order.
13. Incubate at RT for 30 minutes(25-30°C). (Avoid exposure to light).
14. Stop the reaction by adding 100 µl of the stop solution to each well in the same order.

Reading of the results: Read the absorbance at 450nm/630nm on an ELISA reader within 30 minutes.

VALIDATION CRITERIA:

Blank value should be lesser than 0.150

NEGATIVE CONTROL MEAN (NCx)

Individual negative control values should be less than or equal to 0.250 when the photometer is blanked against air. 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.

CALCULATION OF THE NEGATIVE CONTROL MEAN (NCx)

Determine the mean of the negative control values.

Example :

Negative control Sample No.	Absorbance
1	0.044
2	0.051
Total	0.095

$$Ncx = \frac{\text{Total absorbance}}{2} = \frac{0.095}{2} = 0.047$$

POSITIVE CONTROL MEAN (Pcx)

Pcx value has been defined based on lot to lot follow –up. 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 POSITIVE CONTROL MEAN (PCx)

Determine the mean of the positive control values.

Example :

Positive control Sample No.	Absorbance
1	1.867
2	1.958
3	1.779
Total	5.604

$$Pcx = \frac{\text{Total absorbance}}{3} = \frac{5.604}{3} = 1.868$$

CALCULATION OF THE RESULTS

In the above example, all negative control values are within quality control range and the NCx need not be revised.

In the above example PCx minus NCx is greater than 0.600, thus the technique is acceptable and data should be considered valid.

CALCULATION OF THE CUT-OFF VALUE (C.O.V)

The cutoff value is calculated by multiplying the average absorbance value of the HIV Positive control by 0.1 and adding the cut off factor (0.1) Explained mathematically below as.

Cut off Formula: (0.1xPCx) + 0.1

Example : Positive Control Average Absorbance = 1.868

Cutoff value = (0.1x1.868) + 0.1 = 0.286

RESULTS :

1. Non-Reactive : A test sample is considered to be non-reactive for HIV antibodies if the resulting absorbance value is less than the cutoff value.

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

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.

INTERPRETATION OF RESULTS :

1. Specimens with absorbance value is less than the cut-off value are considered non- reactive by the criteria of the HIV ELISA and may be considered negative for antibodies to HIV. Further testing is not required.
2. Specimens with absorbance value greater than or equal to the cutoff be retested in duplicate (using the original sample) before final confirmation of the result.
3. Initially reactive specimens which do not react in either of the duplicate repeat tests are considered negative for antibodies to HIV. Further testing is not required.
4. Initially reactive specimens which are reactive in one or both of the repeat tests are considered repeatably reactive for antibodies to HIV.
5. Specimens which have been found repeatably reactive are interpreted to be positive for the presence of antibodies to HIV. In most settings it is appropriate to investigate repeatably reactive specimens by additional more specific tests such as Radio-Immunoassay, peptide based neutralization EIA and other peptide based EIAs that are capable of identifying antibodies to specific gene products of HIV. As in any diagnostic enzyme immunoassay, there is a possibility that repeatable reactions may occur for the following reasons.

- Inadequate washing
- Contamination of reaction well with HRP conjugate
- Contamination of substrate solution with conjugate or with oxidizing agents.

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
5. HRP conjugate has not been diluted correctly.	Should be diluted 1/100 (1+99)

POSITIVE CONTROL HAS TOO HIGH ABSORBANCE VALUES

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 as indicated

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 low	Volume should be as indicated
3. The volume of sample diluent is too high.	Volume should be 100 µl

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. HRP solution has not been diluted correctly.	Should be 1/100 (1+99)

ALL ABSORBANCE VALUES VERY LOW

Cause/Error	Remedy
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. Deterioration of reagents	Mix the plate before measuring Use aseptic technique. Do not pour used reagent back to vials.
7. 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. Never pour used reagent back to vial.

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 has not been re dissolved before diluting.	Re dissolve 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

POOR SENSITIVITY

Cause/Error	Remedy
1. Too high positive value	See positive control validation criteria.
2. Sample serum or plasma is not mixed properly with sample buffer	While pipetting mix the sample with sample buffer
3. Frozen samples have not been mixed properly after thawing	Mix well before pipetting
4. Stop solution has not been mixed properly before measurement	Mix the plate before measuring

	Intra-assay Variation			Operator-to-Operator Variation		
	Mean (A450nm)	SD	CV%	Mean (A450nm)	SD	CV%
NC	0.049	0.001	2.04	0.051	0.002	3.92
PC	2.383	0.010	0.41	2.387	0.028	1.17

PERFORMANCE CHARACTERISTICS :

Accuracy : The Pareekshak® HIV 1/2ELISA meets the requirement for the third generation test when tested against approved kits.

A. Precision

The reproducibility of pareekshak® HIV1/2 ELISA kit was checked by - assaying three specimens.

Intra-assay

The intra-assay variation of Pareekshak® HIV1/2 ELISA was determined by assaying three samples (Two positive and one negative) in replicates of 16 in a single run.

Serum sample	Mean (O.D)(A_{450nm})	Standard Deviation (SD)	Coefficient of Variation (%)
A	2.377	0.092	3.87
B	1.444	0.039	2.70
C	0.097	0.005	5.15

Table 1 : Summary of the Intra-assay variation study of HIV1/2 ELISA

INTER-ASSAY

The inter-assay variation of Pareekshak HIV1/2 ELISA was determined by assaying three samples (Two positive and One negative) in duplicate of 15 different runs.

Table 2 : Summary of the Inter-assay variation study of HIV1/2 ELISA

Serum sample	Mean (O.D)(A_{450nm})	Standard Deviation (SD)	Coefficient of Variation (%)
A	2.351	0.093	3.95
B	1.461	0.044	3.01
C	0.098	0.005	5.10

B. Sensitivity :

No. of Positive Samples tested	No. of Positives by Pareekshak® HIV 1/2 ELISA Test	Sensitivity (%)
HIV-1 62	62	100
HIV-2 52	52	100

C. Specificity :

No. of Negative Samples tested	No. of Negatives by Pareekshak® HIV 1/2 ELISA Test	Specificity (%)
150	149	99.32

VALIDATION :

Please refer to the schedule below for quality performance as tested with Boston Biomedica Inc. HIV Seroconversion panel PRB 932 and HIV Group O-JP8 & HIV-2,NIH Reference Panel No. 409 has been used.

BBI Panel Member	Result	Abbott ELISA Test	NIH No.	HIV - 2 Status	Result with Pareekshak® HIV 1/2 ELISA
03	-	-	1493	+	+
04	-	-	1494	+	+
05	+	+	1495	+	+
06	+	+	1496	+	+
07	+	+	1497	+	+
08	+	+	1498		
09	+	+			
Group (O) 2701-001	+	+			

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. Sarangadharan MG,Markham PD: The role of human Tlymphotrophic retrovirus in leukemis and AIDS, in ormser GP(ed):AIDS and other manifestations of HIV infection. New Jersey, Noyes Publications,1987,pp218-220.
2. Barre-Sinoussi F.Chermann JC,Rey F.et al.Isolation of Tlymphotrophic retrovirus from a patient at risk for Acquired Immune Deficiency Syndrome (AIDS). Science 220:868-871,1983.
3. Gallo RC, Salahuddin SZ,Popovic M, et al: Frequent detection and iaolation of cytopathic retroviuses (HTLV-III_ from patients with AIDS. Science 224:500-503,1984.
4. Coffin J, Hasse A, Levy, et al: What to call the AIDS virus? Nature 321:10,1986. 5. Clavel F, Guetard D, Brun Vezinet F: Isoaltion of a new human reterovirus from west African patients with AIDS. Science 233:343- 346,1986.

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

2℃ 8℃	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 Reuse	Σ	Sufficient for		KEEP AWAY FROM SUNLIGHT
	KEEP DRY	NON STERILE	NON-STERILE	CONTROL −	NEGATIVE CONTROL
CONTROL +	POSITIVE CONTROL				

R-5, 2013-05-04

QUICK PROCEDURAL REFERENCE:

Addition of Dilution Buffer And Controls (RTU)	100 µl
Label, Add & Patient samples	10 µl
Cover the plate & incubate	30 mins. at R.T (25-30 degrees)
Wash	5 Cycles
Prepare HRP Working conjugate	Strips No 1 2 3 4 5 6 7 8 9 10 11 12 HRP Conc. (µl) 10 20 30 40 50 60 70 80 90 100 110 120 HRP Diluent (ml.) 1 2 3 4 5 6 7 8 9 10 11 12
Add Conjugate	100 µl
Cover the plate & incubate	30 min at R.T. (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
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 100 µl of Ready to use controls (NC & PC Dilutions not required)
↓
Add 100µl of Dilution buffer and 10 µl of Samples
↓
Incubate for 30 min at RT (25-30 degrees)
↓
Wash 5 times with Working wash buffer
↓
Add 100 µl of working HRP conjugate
↓
Incubate for 30 min at RT (25-30 degrees)
↓
Wash 5 times with Working wash buffer
↓
Add 100 µl of Working TMB Substrate
↓
Incubate for 30 min at RT at (25-30 degrees)
↓
Add 100 µl of Stop Solution
↓
Read Absorbance at 450/630nm

Quick calculative information for Programing Elisa readers:

One Blank, 2 NC, 3 PC

Validation:

Blank less than 0.15

Ncx Less than 0.25

Pcx above 0.6

Cut off Formula: (0.1 x PCx) + 0.1

Filters: 450nm/620-630nm

Manufactured in India by :
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