HCV ELISA TEST

For Professional Use

HEPA-SCAN®

An ELISA Test for the detection of antibodies to HCV in Human serum or plasma HCV Antigens for CORE, NS3, NS4 and NS5

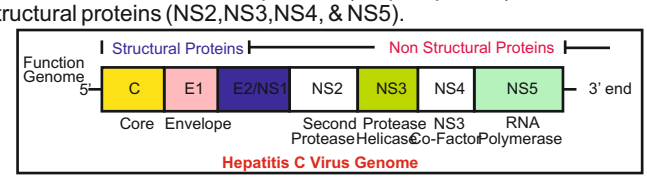
IVD

READ THE PACK INSERT BEFORE USE PROVIDED ALONG WITH THE KIT

REF : HCEL

INTENDED USE : The Hepa-Scan® HCV ELISA Test is for the in vitrodetection of antibodies to Hepatitis C Virus (HCV) in human serum or plasma. It is intended as a screening test for donated blood to prevent transmission of HCV to recipients and as an aid in clinical diagnosis of HCV related infections.

INTRODUCTION : Hepatitis C Virus (HCV) is a small, enveloped, positive-sense single stranded RNA virus. HCV is known to be the major cause of parenterally transmitted non-A,non-B,(NANB) hepatitis. Like the hepatitis B virus, HCV is typically transmitted parenterally. It is associated especially with the transfusion of contaminated blood and blood products. Other common routes of transmission include intravenous drug abuse and needle stick accidents. Present evidence indicates that sexual route is not important in the transmission of HCV. However, HCV transmission occurs more readily and with greater frequency if the sexual partner is co-infected with HIV. Prenatal transmission of HCV from mother to infant is uncommon. The risk of mother to infant transmission may be much greater if the mother is co-infected with HIV. The risk of post transfusion
hepatitis was estimated to be 7 to 18%, with approximately 90% of post-transfusion hepatitis being caused by the NANB hepatitis agent. Conventional methods failed to isolate the virus in cell culture or visualize it by electron microscope. Cloning the viral genome made it possible to develop serological assays that use recombinant antigens. Compared to the first generation HCV ELISA's using recombinant antigen, multiple antigens using recombinant protein and synthetic peptides have been added in new serological tests(Third generation tests) to avoid non specific cross reactivity and to increase the sensitivity of the HCV antibody tests. The test for antibodies to HCV was proved to be highly valuable in the diagnosis and study of the infection, especially in the early diagnosis of HCV after transfusion. The diagnosis of hepatitis C can be easily made by finding elevated serum ALT levels and presence of anti-HCV in serum or plasma. The HCV genome encodes for three structural proteins (Capsid protein) and several non-structural proteins (NS2,NS3,NS4, & NS5).



Hepatitis C Virus Genome

This third generation antibody test uses a greater range of antigens from Core, NS3, NS4,& NS5 regions of the HCV genome allowing the detection of specific antibodies to multiple viral epitopes and thus providing greater sensitivity and better specificity. In addition, the use of these additional antigens allows earlier detection of antibodies during seroconversion following HCV infection.

Hepa-Scan® HCV ELISA Test utilizes a unique combination of HCV antigens from the putative core, NS3, NS4,& NS5 regions of the virus to selectively detect all sub types of Hepatitis C Virus in human serum or plasma with a high degree of sensitivity and specificity.

Hepa-Scan® HCV ELISA Test is an immunoassay which employs recombinant proteins and synthetic peptides for the detection of antibodies to HCV in human serum or plasma. These peptides and recombinant proteins, which corresponds to highly antigenic segments of both the structural and non-structural proteins of the hepatitis C virus, constitute the solid phase antigenic absorbent. The use of synthetic peptides and recombinant proteins offers the advantage of increased sensitivity and specificity.

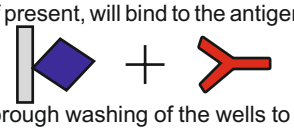
PRINCIPLE :

Hepa-Scan® HCV ELISA is an Indirect antibody EIA assay for detection of antibodies to HCV virus in Human Serum or plasma.

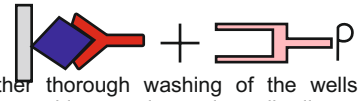
1. Hepa-Scan® HCV ELISA Test employs an immunosorbent enzyme assay, which consists of recombinant protein for Core and NS3 protein and synthetic peptides corresponding to highly antigenic segments, NS4 and NS5 regions of the hepatitis C virus, bound to the wells of the microplate.


Antigen bound to microwell

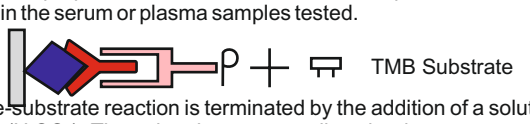
2. During the course of the assay, diluted controls and diluted specimens are added to the wells and incubated. HCV specific antibodies, if present, will bind to the antigens.


Antibody

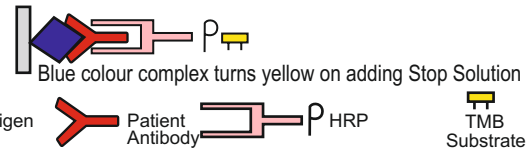
3. After a thorough washing of the wells to remove unbound antibodies and other serum components, a standardized preparation of horseradish peroxidase-conjugated is added to each well. This conjugate preparation is then allowed to react with antibodies which bind to the assay wells on the basis of their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


HRP

4. After another thorough washing of the wells to remove unbound horseradish peroxidase-conjugated antibodies, a substrate solution containing hydrogen peroxide (H_2O_2) and TMB is added to each well. A blue color develops in proportion to the amount of HCV 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.



Schematic Representation of Hepa-Scan® HCV ELISA

STORAGE AND STABILITY :

STORAGE :

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.

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)	12ml	15ml	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)
10.Disposable latex gloves.

SPECIMEN COLLECTION AND HANDLING :

Specimens must be centrifuged before use (e.g. 3000 RPM, 10 minutes), especially citrate plasma specimens will cause false HCV 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 HCV reactive results.

Do not use sodium azide as preservative because it inactivates horseradish peroxidase. Microbially-contaminated, grossly hemolysed, icteric or rlipemic serum and plasma specimens may give erroneous results.

PRECAUTIONS:

1. For in vitro diagnostic use only.
2. The positive control contains inactivated HCV 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 “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 o 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 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 & 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.
2. Preparation of Enzyme Conjugate:
Dilute 100X HRP conjugate diluent. For eg. For 8 wells mix 10µl of 100X HRP conjugate and 1 ml of HRP conjugate diluent.
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.
Preliminary preparations:
1. Wear disposable latex gloves throughout the procedure.
2. Bring all reagents and Microwells to Room temperature (25- 30°C) before starting the assay. Gently mix all liquid reagents before use.

TEST PROCEDURE:

1. Set up the microtitre wells in the frame provided and label each well. Label one well(A1) as blank and two (B1 & C1) wells as Negative and Three wells (D1, E1 & F1) as Positive controls.
2. Add 100µl of Positive and Negative controls (READY TO USE) to appropriately labeled wells of the microtiter plate.
3. Add 100 µl of dilution buffer into respective number of wells other than Controls assigned.
4. Add 10µl of test 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. Wash the microplate 5 times with approximately 300µl per well of working wash solution. Care should be taken to avoid overfilling and cross contamination.
8. Add 100µl of the working HRP conjugate solution in the same order.
9. Incubate at RT for 30 minutes (25-30°C).
10. Wash the microplate 5 times with approximately 300 µl per well of working wash Solution
11. Add 100µl of working TMB substrate to each well in the same order.
12. Incubate at RT for 30 minutes(25-30°C). (Avoid exposure to light).
13.Stop the reaction by adding 100ul of the stop solution to each well in the same order.
14).Reading of the results : Read the absorbance at 450nm/630nm on an ELISA reader within 30 minutes.
NOTE : The reference wavelength of spectrophotometer could be 620nm to 650nm. However user should validate the spectrophotometer in combination with this before use.

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 RESULTS

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

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

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

CALCULATION OF THE POSITIVE CONTROL MEAN (PCx)

Determine the mean of the positive control values.

Example :

Positive control Sample No.	Absorbance
1	1.432
2	1.434
3	1.431
Total	4.296

$$Pcx = \frac{\text{Total absorbance}}{3} = \frac{4.296}{3} = 1.432$$

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.)

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

Cut off Formula: (0.1xPCx) + 0.1

Example : Positive Control Average Absorbance =1.432
Negative Control Average Absorbance = 0.047
Cutoff value = (0.1x1.432) + 0.1 = 0.143

RESULTS :

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

2. Reactive : A test sample is considered to be reactive for HCV 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 HCV ELISA and may be considered negative for antibodies to HCV. Further testing is not required.
2. Specimens with absorbance value greater than or equal to the cutoff value are considered initially reactive. These specimens should 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 HCV. Further testing is not required.
4. Initially reactive specimens which are reactive in one or both of the repeat tests are considered repeatable reactive for antibodies to HCV.
5. Specimens which have been found repeatably reactive are interpreted to be positive for the presence of antibodies to HCV. 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 HCV. 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 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

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 :

The Hepa-Scan® HCV ELISA meets the requirement for the third generation test when tested against approved kits.

A. Precision

The Intra-assay variation of Hepa-Scan® HCV ELISA 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 1. Summary of the intra-assay and Operator-to Operator variation study of Hepa-Scan® HCV ELISA Test.

	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

Inter-assay

The inter-assay variation of Hepa-Scan® HCV ELISA Test was determined by testing negative and positive samples in 10 independent test runs. Summary of the results is presented in table 2.

Table: 2 Summary of the Inter-assay variation study of Hepa-Scan® HCV ELISA.

B. Sensitivity :

No. of Positive samples tested	No. of Positive by HEPA-SCAN® HCV Test	Sensitivity (%)
105	104	99%

C. Specificity :

No. of Negative samples tested	No. of Negative by HEPA-SCAN® HCV Test	Specificity (%)
200	199	99.5%

Dilution Series Test

Samples Dilution	Result OD 450nm
Nil	2.533
1:100	0.582
1:1000	0.483
1:2000	0.286

KIT PERFORMANCE :

Please refer to the schedule below for quality performance as tested with Boston Bio medica Inc., HCV low titer performance panel PHV

Panel Member	Result	HCV 3.0 (Abbott)
01	+	+
02	++	++
03	+	+
04	+	+
05	++	++

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. NCCLS Document M29-T2 (1991). Protection of laboratory workers from instrument biohazards. Vol. 11,No 15.
2. Caypers, H.T.M. Wiakel, I.N.Vander Poel, C.L. et al.((1971) J. of Hepatology, 13,5.15.
3. Halfon, P.etal(1997) J. Medical Virology. 52:391-395.
4. Sarin, S.K. & Hess. G. (1998). Transfusion associated Hepatitis.
5. Sayers, M.H. & GretchD.R. (1993). J. Transfusion 30,809-13.

QUICK PROCEDURAL REFERENCE:

Addition of Dilution Buffer and Ready to use Controls	100 µl
Label, Add & Patient samples Respective wells	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 100µl of Dilution buffer and Ready to use controls

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Add 10µl of patient Samples

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Incubate for 30 min at RT (25-30 degrees)

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Wash 5 times with Working wash buffer

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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

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				

R-5, 2013-05-05

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