HBsAg ELISA TEST

For Professional Use

HEPA-SCAN®

A solid-phase enzyme immunoassay for the detection of Hepatitis B Surface Antigen (HBsAg) in human serum or plasma

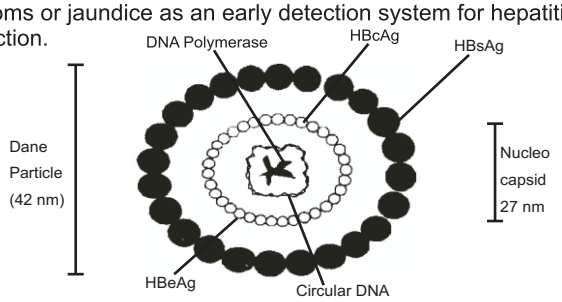
IVD

READ THE PACK INSERT BEFORE USE PROVIDED ALONG WITH THE KIT

REF : HBEL

INTENDED USE : HEPA-SCAN® HBsAg ELISA Test is an in vitro enzyme immunoassay for the detection of hepatitis B surface antigen in human serum or plasma. The HBsAg ELISA is primarily used for testing blood and organ donors, patients with active viral hepatitis and people at risk for hepatitis B infection.

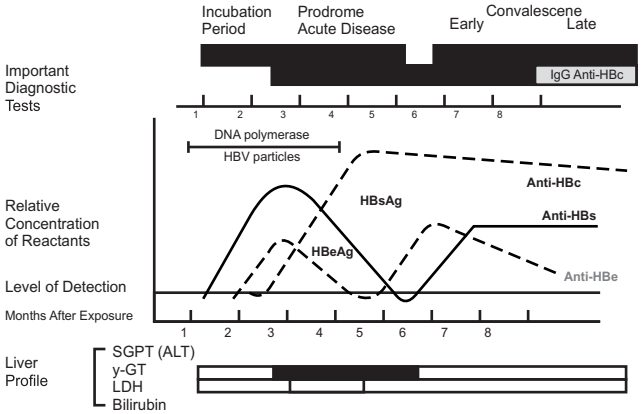
INTRODUCTION : Hepatitis B virus is responsible for the most serious form of the hepatitis related diseases. HBV is transmitted through sexual contact blood and other body fluids transmitted from an infected mother to her child during the perinatal period. Hepatitis B Surface Antigen (HBsAg) is usually detectable around two to six weeks in advance of clinical and biochemical evidence of hepatitis and persits throughout the clinical course of the disease. Level of HBsAg is of maximium titer at the height of liver damage. HBsAg is a complex lipid and glycoprotein particle containing group specific determinants 'd/y' and 'w/r'. Thus HBsAg can be subdivided into four major antigenic subtypes : 'adw', 'adr', 'ayw', and 'ayr'. The subtype 'ayw' is predominant in India. Hepatitis B Virion (HBV) or Dane particle is a double-stranded virus which is 42nm in diameter with a lipoprotein coat and a nucleocapsid core. The lipoprotein coat is composed of Hepatitis B surface antigen (HBsAg). The nucleocapsid is 27nm in diameter and possesses a distinct antigenic specificity (HBcAg) along with a partially double stranded DNA molecule and an endogenous polymerase enzyme. Another antigen called "Hepatitis B" e antigen (HBeAg) is a split protein of HBcAg. All the above antigens and viral DNA polymerase form useful diagnostic markers for HBV. However, HBsAg has been accepted as a universal and the most reliable seromarker in case of acute HBV infection due to its utmost appearance almost 2-4 weeks before the ALT level becomes abnormal and 3-5 weeks before the onset of symptoms or jaundice as an early detection system for hepatitis B virus infection.



Structure of Hepatitis B virus

CLINICAL SIGNIFICANCE :

In most cases of Hepatitis B infection incubation period varies from 40 days to 6 months, but is around two to three months on an average. A prodormal illness occurs in some patients who complain of malaise and anorexia accompanied by weakness and myalgia. Anthralgia also occurs and may be accompanied by an utricarial or maculopapular rash. The diagnosis of Hepatitis B infection depends on finding components of virus and specific antibodies in the blood.



SEROLOGICAL & CLINICAL PATTERN DURING ACUTE HBV INFECTION

PRINCIPLE :

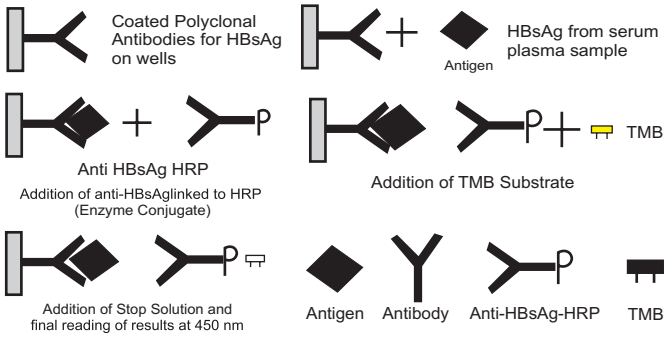
HEPA-SCAN® HBsAg ELISA is a solid phase Enzyme linked immunosorbent assay based on sandwich capture principle.

1.When patients serum containing HBsAg is added, it will combine with the goat Anti-HBsAg attached to the polystrene surface of the Microwells and simultaneously bind with the horseradish peroxidase conjugated Monoclonal Anti-HBsAg.

2.Wells are washed and a colour less enzyme substrate (H_2O_2) and chromogen (TMB, Tetramethylbenzidine) are added. The enzyme acts on substrate/chromogen producing a blue colored end product.

3.The enzyme substrate/chromogen reaction is terminated with acid (H_2SO_4). The yellow color intensity is directly related to the concentration of hepatitis B surface antigen in the patient sample.

Schematic Representation of "HEPA-SCAN® HBsAg 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, 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 Tests	96 Tests	480 Test
Anti-HBsAg coated microwells (Ready to use)	8 wells x 6 strips	8 wells x 12 strips	96Wells x 5plats
Wash solution (Concentrated 10X)	25ml	50ml	5x50 ml
Anti-HBs HRP Conjugate (50x Conc)	0.075 ml	0.150 ml	5x0.150 ml
Anti HBs HRP Conjugate Diluent	4 ml	8 ml	5x8 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	1 No.	2 Nos.	10 Nos.
Pack Insert	1No.	1No.	1No.

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 HBsAg 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 at least 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 HBsAg reactive results.

Do not use sodium azide as preservative because it inactivates horseradish peroxidase. Microbially-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 HBsAg 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 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 Working Enzyme (BEFORE USE ONLY):

Mix Anti HBsAg Enzyme Concentrate and Anti HBsAg HRP Diluent in 1:50 ratio to prepare working Enzyme. for eg :

For 8 Wells Mix 0.5 ml of Enzyme Diluent and 0.10 µl of Anti HBs HRP Conjugate 10 l of TMB Substrate and 0.5 ml of TMB Diluent.

NOTE : Prepare working Substrate solution freshly every time mix solution thoroughly before use.

3.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 solutions throughly 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. 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.
4.Set up microtitration 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 microtiter plate.
7.Add 50 µl of Working Anti-HBs HRP conjugate solution to each well EXCEPT BLANK and mix throughly by gentle swirling
8.Cover the wells with adhesive slips.
9.Incubate at 37°C for 60 minutes.
10.Wash the microplate 5 times by adding 300 µl (approximately) of working wash solution.
11. Add 100µl of working substrate solution including blank.
12.Incubate at RT for 30 minutes (25°C to 30°C).Avoid expose to light
13. Add 100µl of stop solution to each well including blank.
14. 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 0.20

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
1	0.038
2	0.030
Total	0.068

Ncx = $\frac{\text{Total absorbance}}{2} = \frac{0.068}{2} = 0.034$

Positive control Sample No.	Absorbance
1	2.523
2	2.505
3	2.490
Total	7.518

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 HBsAg if the resulting absorbance value is less than the cut-off value.

2. Reactive :A test sample is considered to be reactive for HBsAg 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 HEPA -SCAN® HBsAg ELISA test and may be considered negative for HBsAg. Further testing is not required.
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 HEPA-SCAN® HBsAg ELISA Test. 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 HBsAg. Further testing is not required.

b)Initially reactive specimens which are reactive in one or both of the repeat tests are considered repeatedly 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 HBsAg 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 HBsAg.

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

HEPA-SCAN® HBsAg ELISA meets the requirement for the third generation test when tested against the FDA/DCI approved kits.

HBsAg ELISA was tested with the HBsAg subtype panel consisting of Ad/Ay subtypes: HBsAg positive with HEPA-SCAN® HBsAg ELISA Test.

A. Precision Intra-assay

The intra-assay variation of HEPA-SCAN® HBsAg ELISA was determined by testing positive and negative samples containing HBsAg.

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 varation study of HEPA-SCAN® HBsAg ELISA 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 HEPA-SCAN® HBsAg ELISA Test	Sensitivity (%)
99	98	99 %

No. of Negative Samples tested	No. of Negatives by HEPA-SCAN® HBsAg 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	HBsAg Test (Abbott)	HEPA-SCAN® HBsAg 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 :

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2.Blumberg. B.S., Alter, H.J. and Visnich, S.A. "New" Antigen in leukemia sera, J.A.M.A. 191: 541-546, 1965.
3. Voller, A., Bartlett, A. and Bid well, D.E. Enzyme Immunoassays with Special Reference to ELISA Techniques, J.Clin. Pathol. 32: 507-520, 1978.
4.Fields,H.A., Devis, C.L.,Bradley, D.W. and Maynard, J.E.Experimental conditions affecting the sensitivity of Enzyme Linked Immunosorbent Assay (ELISA) for detection of Hepatitis B Surface Antigen (HBsAg).Bull. W.H.O.61: 135-142, 1983.

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 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		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 Enzyme Conjugate		50 µl
Cover the plate & incubate		60 min at 37°C 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 °C 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 Anti HBs HRP conjugate
↓
Incubate 60 minutes at 37°C
↓
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°C 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 0.20
Ncx Less than 0.25
Pcx above 0.6
Cut off Formula: NCx + 0.1
Filters: 450nm/620-630 nm

R-5, 2013-05-05

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