VANSCAN™

# HBsAg Microwell ELISA

## Enzyme immunoassay for the detection of HBsAg in Human Serum or Plasma

### INTENDED USE

HBsAg Microwell ELISA is an *in vitro* enzyme immunoassay for the qualitative detection of Hepatitis B surface Antigen in Human Serum or Plasma.

### INTRODUCTION

Hepatitis B Virus (HBV) has been shown to be the viral agent responsible for Hepatitis B and has been linked to the development of primary hepatocellular carcinomas.

The presence of a component of the HBV, known as the Hepatitis B surface Antigen (HBsAg) in the serum, indicates either a chronic or acute infection with the virus.

A number of assays for HBsAg are available, many of which use polyclonal Anti-HBs antibodies on the solid phase.

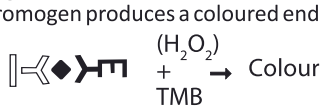
HBsAg Microwell ELISA kit represents a new generation of HBsAg assays in which specific monoclonal Anti-HBs antibodies are used. This assay promises to be more sensitive, convenient and safer to use, and has the advantage of short reaction time as well as easier detection of target materials.

### PRINCIPLE OF THE TEST

The principle of HBsAg Microwell ELISA is based on a direct, noncompetitive, solid-phase enzyme immunoassay with Horse Radish Peroxidase as the marker enzyme. The assay proceeds according to the following reactions:

1. HBsAg ( ♦ ) when present in patient's serum, combines with the mouse monoclonal anti-HBs (⋖ ) antibodies coated on the polystyrene surface (‖) of the microstrip wells and simultaneously binds with the Horse Radish Peroxidase conjugated goat poly anti-HBs ( ⋗ ) antibodies.

2. After incubation, wells are washed and a colourless substrate ($H_2O_2$)chromogen(TMB)' solution is added. The enzyme action on substrate/chromogen produces a coloured end product.




3. The enzyme-substrate/chromogen reaction is terminated with addition of the Stop Solution. The colour intensity is directly related to the concentration of Hepatitis B surface Antigen in the patient sample.

### KIT CONTENTS

1. **HBsAg Antibody Coated Microplate/Microstrips**
   One plate of 96 microwells coated with monoclonal anti-HBsAg Antibody. Allow the wells to reach room temperature (18 to 30°C) before removal from the bag. Place unused wells in the sealable storage bag provided and return to 2-8°C. Once, opened, microwells should be used within one month.
2. **Sample Diluent**
   One bottle containing buffered solution containing proteins, stabilizer, preservative and indicator dye for sample addition.
3. **Negative Control**
   One vial containing 0.5 ml of normal human serum with preservative. Negative Control has been tested and found to be negative for anti-HIV 1+2, HBsAg, Anti-HCV and Syphilis.
4. **Positive Control**
   One vial containing 0.5 ml of inactivated human serum in a buffer containing protein with preservatives. Positive Control has been tested and found to be negative for anti-HIV 1+2, Anti-HCV and Syphilis.
5. **Conjugate Concentrate (101X)**
   One vial containing polyclonal Anti-HBsAg conjugated to HRP with protein stabilizers and preservatives. For preparation of working reagents, refer to Step wise Procedure Card provided along with this Kit.
6. **Conjugate Diluent**
   One bottle containing solution consisting of buffer, protein, preservatives and detergent. For preparation of Working Conjugate Solution, refer to Step wise Procedure Card provided along with this Kit.
7. **TMB Substrate (101x):**
   One vial containing 0.3ml of 3,3',5',5'- Tetramethyl benzidine - TMB, (101x) containing Dimethyl Sulfoxide (DMSO) as solvent.
8. **Substrate Buffer:**
   One bottle containing 15ml of Substrate Buffer containing Hydrogen Peroxide ($H_2O_2$) and Stabilizers. For Preparation of Working TMB Substrate solution, refer to the preparation of Working Reagents.
9. **Wash Solution Concentrate (20X )**
   One bottle containing 20 times working strength phosphate buffer saline Wash Solution with detergent. For preparation of Working Wash Solution, refer to Step wise Procedure Card provided along with this Kit.
10. **Stop Solution**
    One bottle containing colourless solution of diluted acid and stabilizers.
11. **Microstrip Covers**
    Sheets to seal microstrips during incubation.
12. **Package Insert**
13. **Procedure Card**

### MATERIALS REQUIRED BUT NOT PROVIDED

(A) Distilled or deionized water, (B) Graduated Cylinder for reagents dilutions, C) Micropipettes, (D) Paper towels or absorbent paper, (E) Timer, F) Incubator, maintained at 37±1°C, (G) ELISA Reader, (H) ELISA Washer, (I) Sodium hypochlorite solution (free available chlorine 50-500mg/dl) and J) Disposable gloves.

### SPECIMEN TRANSPORT AND STORAGE

Store the samples at 2-8°C. Samples not required for assay within 7 days should be stored frozen (-15 °C or colder). Avoid multiple freeze-thaw cycles. After thawing ensure samples are thoroughly mixed before testing.

### PRECAUTIONS

1. Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated area swabbed with, for example, 1.0% Sodium Hypochlorite before work is continued. Sodium Hypochlorite should not be used on acid-containing spills unless the spill area is first wiped dry. Materials used to clean spills, including gloves, should be disposed off as potentially Bio-Hazardous waste. Do not autoclave materials containing's Sodium Hypochlorite.
2. Do not pipette by mouth. Wear disposable gloves and eye protection while handlings specimens and performing the assay. Wash hands thoroughly thereafter.
3. Sulphuric acid used in Stop Solution is corrosive and should be handled with appropriate care. If it comes into contact with skin or eyes, wash thoroughly with water. If any of the reagents come into contact with skin or eyes wash the area extensively with water.
4. Do not use the reagents beyond the stated expiry date.
5. Do not modify the test procedure or substitute reagents from other manufactures or other lots unless the reagents are stipulated as interchangeable.
6. Do not reduce any of the recommended incubation times.
7. Allow the reagents and samples to come to 18 to 30°C before use. Immediately after use, return reagents to the recommended storage temperature.
8. Do not allow wells to become dry during the assay procedure.
9. Do not cross-contaminate reagents. It is recommended to use dedicated separate pipettes for dispensing the Substrate Solution and Conjugate.

10. Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate.

**PREPARATION OF TEST**

1. Bring all the reagents to room temperature 30 minutes before use.
2. Take the required number of strips from the sealed coated plate. The remaining strips must be kept at 2-8°C with the silica gel after proper sealing of the pouch.

**TEST PROCEDURE**

1. Take the required number of strips and fix them on to the plate.
2. Pipette 50µl of Sample Diluent into each plate well except Blank well A1 and pipette 50 µl of Negative Control into each well from B1 to D1 and 50 µl of Positive Control into each well from E1 to F1, respectively, and then pipette 50 µl of each sample into the remaining wells.
3. Incubate at 37±1°C for 30 minutes after sealing the plate with the Microstrip Covers.
4. Before the last 5-10 minutes of the 1st incubation, make a 1:100 dilution of the Conjugate with Conjugate Diluent. Refer to the Step Wise Procedure Card provided along with this kit.
5. Aspirate the contents from each of the wells and wash each well 5 times with 350µl of Working Wash Solution. Refer to the Step Wise Procedure Card provided along with this kit.
6. Invert the plate and tap it on absorbent paper to remove the remaining Wash Solution and then pipette 100µl of prepared Working Conjugate Solution into each well except Blank well A1.
7. Incubate the plate at 37±1°C for 30 minutes after sealing it with the Microstrip Covers.
8. Aspirate the contents from each of the wells and wash each well 5 times with 350µl of Working Wash Solution.
9. Invert the plate and tap it on absorbent paper to remove the remaining Wash Solution and then pipette 100 µl of working Substrate Solution into each well including the Blank well A1. Refer to the Step Wise Procedure Card provided along with this kit.
10. Incubate it at controlled room temperature (21-25°C) for 30 minutes.
11. Pipette 50µl of Stop Solution into each well including Blank well A1.
12. Read the absorbance at 450nm (reference wavelength at 630nm) within 15 minutes after pipetting the Stop Solution.

**QULITY CONTROL**

Results of an assay are valid if the following criteria for the Controls are met:-

**a)** **Absorbance of Blank** should be **< 0.050.**

**b)** **Mean Absorbance of Negative Controls** should be **< 0.100**. If absorbance of Negative Control comes in negative. eg. - 0.003 it should be considered as zero.

**c)** **Mean Absorbance of Positive Controls** should be **> 1.000.**

If the results are outside the above ranges, the test should be conducted again.

**CUT OFF VALUE:**

Calculations of the Cut off Value (COV) :

(a) Calculate the Negative Control mean ($NC\bar{x}$)

e.g.) Negative Control 1 absorbance = 0.009

Negative Control 2 absorbance = 0.010

Negative Control 3 absorbance = 0.011

Negative Control Mean ($NC\bar{x}$) =

(0.009+0.010+0.011)/3 = 0.010

(b) Calculate the Cut off Value (COV) :

Cut off Value (COV) = $NC\bar{x}$ +0.100 = 0.010 + 0.100 = 0.110

**INTERPRETATION OF RESULTS**

**Reactive Results: -** Samples with absorbances greater than or equal to the Cut off Value are considered Reactive in the assay.

**Non-Reactive Results: -** Samples with absorbances less than the Cut off Value are considered non-reactive in the assay.

**NOTE:-** If the samples are considered reactive, the test should be conducted two more times.

**Gray zone samples** : whose absorbance falls in between ±10% of Cut off Value.

In cases where the re-tests show non-reactive, the samples are considered non-reactive, and on the other hand, if one of the re-tests shows reactive, the samples are considered reactive.

The samples considered reactive should be tested again by the Neutralization Assay for final interpretation.

**PERFORMANCE CHARACTERISTICS**

| | |
|---|---|
| Analytical Sensitivity | 0.4 PEIU/ml |
| Diagnostic Sensitivity | 100% |
| Diagnostic Specificity | 100% |

**BIBLIOGRAPHY**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