HBsAg ELISA Kit

Prod. No.: DEIA511
Pkg. Size: 96T

INTENDED USE

HBsAg ELISA Kit is an in-vitro diagnostic test for the detection of Hepatitis B Virus Surface Antigen in human serum. For professional use only.

GENERAL DESCRIPTION

HBsAg ELISA Kit is an Enzyme-Immunoassay (EIA) for the detection of Hepatitis B Surface Antigen (HBsAg) in human serum. Acute Viral Hepatitis, caused by the Hepatitis B Virus, is a common and serious infection. It is characterised by inflammation and necrosis of the liver and with the exception of chronic liver disease, it is the single most significant cause of mortality by liver cancer. Transmission of Hepatitis B Virus occurs by direct human transmission via parental routes such as infective serum, blood, blood transfusion and contaminated needles or by non-parental transmission through body fluids such as saliva, urine and semen. Tests to detect HBsAg are now widely used for the detection of infected blood products, infected patients and healthy carriers of the disease. Pathozyme HBsAg is classified as a Third Generation test according to the specifications of the US. F.D.A.

PRINCIPLE OF THE TEST

Monoclonal antibodies, specific for the eight known HBsAg subtypes, recognised by the W.H.O., are bound to the surface of microtitration wells. Undiluted test serum are added followed by Anti-HBsAg antibody conjugated to Horseradish Peroxidase (HRP). If HBsAg is present in the sample it binds to the antibody in the wells and the Conjugate binds to the captured viral antigens. If HBsAg is not present binding does not take place and unbound material is washed away. On addition of the Substrate, stabilised 3, 3', 5, 5' Tetramethyl Benzidine (TMB), a colour will develop only in those wells in which the HRP is present, indicating the presence of HBsAg. The reaction is stopped by the addition of dilute Sulphuric Acid and the absorbance is then measured at 450nm. Any result with an optical density (OD) greater than the cut-off should be considered positive.

REAGENTS PROVIDED

1. Microtitre Plate(12x8): Breakable wells coated with monoclonal antibody contained in a resealable bag with a desiccant
2. Control(2ml): Negative Control. Clear solution of human serum negative for HBsAg. Ready for use. (Blue)
4. Wash Buffer concentrate(50ml): Tris based buffer containing detergents. (Colourless)
5. Anti-HBsAg HRP(11ml): Anti-HBsAg Conjugated to Horseradish Peroxidase. Ready for use. (Purple)
7. Stop Solution(11ml): Sulphuric Acid diluted in purified water. Ready to use. (Colourless)

STORAGE

1. Reagents must be stored at temperatures between 2°C to 8°C.
2. Expiry date is the last day of the month on the bottle and the kit label. The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Do not use reagents after the expiry date.
3. Exposure of reagents to excessive temperatures should be avoided. Do not expose to direct sunlight.
4. DO NOT FREEZE ANY OF THE REAGENTS as this will cause irreversible damage.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micropipettes: 100μl, 200μl, 1000μl and 5000μl
2. Disposable pipette tips
3. Incubator: Temperature of 37°C +/- 1°C.
4. Absorbent paper
5. Microplate reader fitted with a 450nm filter
6. Graph paper
7. Thoroughly clean laboratory glassware.

SPECIMEN COLLECTION AND HANDLING

1. Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required.
2. Do not use haemolysed, contaminated or lipaemic serum for testing as this will adversely affect the results.
3. Serum may be stored at 2°C to 8°C for up to 48 hours prior to testing. If longer storage is required, store at –20°C for up to 1 year. Thawed samples must be mixed prior to testing.
4. Do not use Sodium Azide as a preservative as this may inhibit the Peroxidase enzyme system.
5. Do not repeatedly freeze-thaw the specimens as this will cause false results.

Creative Diagnostics. All rights reserved.

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com
CALCULATION

1. For each test and Control Serum determine the Optical Density (OD) obtained in the wells.
2. Cut off level = Average OD of Negative Control + 0.10
3. Assay Validation: The OD of the Positive Control must exceed 0.300
4. The OD of the Negative Control must be lower than 0.15.
5. Negative Result: A negative result should have an OD less than the cut-off. However, results with an OD up to 10% lower than the cut-off value should be considered as equivocal. These samples should be tested again and if the same result is obtained, another assay should be performed after 1-2 weeks. Possible causes for these equivocal results could be contaminated samples, non-specific reactions or samples with HBsAg levels below the cut-off value of the test.
6. Positive Result: A positive result should have an OD equal or higher than the cut-off value. Positive results should be and if the sample continues to be positive, a neutralisation confirmatory test should be performed on that sample.
7. If levels of controls or users known samples do not give expected results, test results must be considered invalid.

ASSAY CHARACTERISTICS

1. The Antibodies used in this kit have been selected to provide a system which will detect small quantities of HBsAg including all eight subtypes of Hepatitis B Virus. Lowering the cut-off value increases the sensitivity but at the expense of specificity with the number of false positives being increased. A factor of 0.1 is used here but it may be preferable to determine the appropriate cut-off factor for the patient population under test.
2. Assay Sensitivity approximately 0.5ng/ml
3. With a cut-off of negative control + 0.1

REFERENCES