DATA SHEET

Immunoassay

REF **E0319**

96 tests

Anti-HBc ELISA

microplate based ELISA (enzyme linked immunosorbent assay) for the qualitative detection of Anti-HBc (antibodies to hepatitis B core antigen) in human serum or plasma.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use

IVD

Introduction

Hepatitits B is a viral infection, in which the route of infection can be sexual contact, improper needle puncture, blood transfusion or even contaminated food or water. HBcAg (hepatitis B core antigen) is a hepatitis B viral protein.[1,2] It is an indicator of active viral replication, which means the person infected with Hepatitis B can likely transmit the virus on to another person (i.e. the person is infectious). The determination of Anti-HBc levels can be sued to examine the progress of HBV (hepatitis B virus) infection.[3] In acute case of hepatitis B infection, Anti-HBc is detectable in the blood shortly after the appearance of HBsAg.[4] HBsAg levels often decline before the appearance of Anti-HBs. During this interval between the decline of HBsAg and the rise in Anti-HBs, total Anti-HBc may be the only reliable marker of HBV infection. In chronic HBV infections, HBsAg rises during the incubation phase and may persist for years. Anti-HBc also appears during this early phase and reaches high titers which may persist for years.[5] In asymptomatic HBV infections, HBsAg and HBeAg are present only briefly and are quickly followed by the appearance of Anti-HBs and Anti-HBc.[6] Therefore in such patients, sometimes the only evidence of an infection may be the detection of Anti-HBs and Anti-HBc.

Measurement Principle

This assay is based upon the one-step competitive method. Sample, recombinant HBcAg coated microwells and enzyme labeled Anti-HBc are combined. During the incubation, enzyme labeled Anti-HBc and Anti-HBc present in the sample compete for binding to the recombinant HBcAg coated on microwells. After washing, a complex is generated between the solid phase and enzyme-linked antibodies by immunological reactions. Substrate A and substrate B are then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance. The color intensity is inversely proportional to the amount of Anti-HBc in the sample.

Components

1. Coated Wells

1 plate of 96 wells pre-coated with recombinant HBcAg.

2. Enzyme Conjugate

1 vial containing 7.5 ml of HRP (horseradish peroxidase) labeled mouse monoclonal Anti-HBc in a buffer solution. Contains 0.1% ProClin 300® preservative.

3. Negative Control

1 vial containing 1 ml of a carbonate buffered solution containing proteins of bovine origin. Contains 0.1% ProClin 300® preservative.

4. Positive Control

1 vial containing 1 ml of a phosphate buffered solution containing heat-inactivated human plasma positive for Anti-HBc and proteins of bovine origin. Contains 0.1% ProClin 300® preservative.

5. Stop Solution

1 vial containing 7.5 ml of 0.62 mol/l sulfuric acid.

6. Substrate A

1 vial containing 7.5 ml of hydrogen peroxide.

7. Substrate B

1 vial containing 7.5 ml of TMB (3, 3', 5, 5'-tetramethylbenzidine) in a buffer solution.

8. Wash Solution Concentrate

1 vial containing 30 ml of 20 times working strength PBS-Tween wash buffer.

9. 1 copy of instruction for use
10. 1 piece of plate lid
11. 1 Zip-lock bag

Materials Required but not Provided

1. absorbent paper or paper towel
2. automated microplate strip washer
3. distilled water
4. disposable reagent troughs
5. incubator
6. magnetic stirrer
7. micropipettes and multichannel micropipettes of appropriate volumes
8. microplate reader
9. plate shaker

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
3. Handle the potentially contaminated materials safely according to local requirement.
4. CAUTION: the positive control contains human sourced component, which has been tested and found non-reactive for HIV 1 and HIV 2 antibodies, Anti-HTLV I & II and HCV antibodies and syphilis, and reactive for Anti-HBc. It is recommended that all human sourced materials be considered potentially infectious. This assay contains materials of animal origin. Bovine components originate from countries where BSE has not been reported.
5. Do not smoke, drink, eat or use cosmetics in the working area.
6. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
7. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
8. Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.
9. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
10. Wash the wells completely. Each well must be fully injected with wash solution. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, dry the liquids in each well. Strike the microplate onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate strip washer.
11. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
12. Do not touch or splash the rim of the well with conjugate. Do not blow out from micropipettes.
13. Do not use reagents beyond the labeled expiry date.
14. Do not mix or use components from kits with different batch codes.
15. When manual pipette is used, complete pipetting of all controls, samples within 10 minutes.
16. It is important that the time of reaction in each well is held constant to achieve reproducible results.
17. It is important to calibrate all the equipment e.g. micropipettes, microplate readers, automated microplate strip washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
18. 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.
19. The addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same se-

quence to eliminate any time deviation during reaction.

20. Do not allow wells to become dry during the assay procedure.

Storage

1. Store all components at 2-8 ℃. Do not freeze. Avoid strong light.
2. Place unused wells in the Zip-lock bag with the desiccant provided, then seal the Zip-lock bag in the aluminum foiled pouch with a plate lid and return to 2-8 ℃, under which conditions the wells will remain stable for 2 months, or until the labeled expiry date, whichever is earlier.
3. Seal and return all the other unused reagents to 2-8 ℃, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Sample

1. Collect samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples. Do not use sodium azide preservatives in samples.
3. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
4. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
5. Insufficient processing of sample, or disruption of the sample during transportation may cause depressed results.
6. Avoid grossly hemolytic, lipemic or turbid samples.
7. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 24 hours. Or freeze the samples that need to be stored or transported for more than 24 hours at -20 ℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
8. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
9. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
10. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.

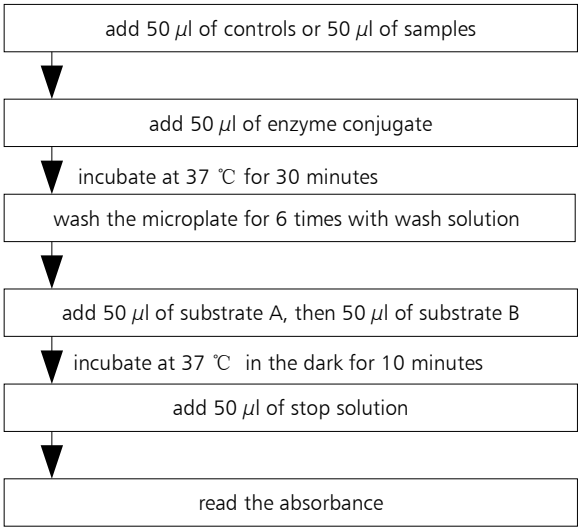
Reagent Preparation

1. Bring all reagents to room temperature (18-25 ℃) prior to use for at least 30 minutes. Mix all reagents through gently inverting prior to use. Do not induce foaming.
2. Adjust the incubator to 37 ℃.
3. Add 1 volume of wash solution concentrate to 19 volumes of distilled water to give the required volume, and mix well with a magnetic stirrer. The wash solution is stable at room temperature for 2 months.

Measurement Procedure

1. Use only the number of wells required and format the microplates' wells for each control and sample to be assayed.
   Leave well A1 as the blank well. To each plate add 50 μl of the negative controls to wells B1, C1 and D1 and 50 μl of positive controls to wells E1 and F1. Add 50 μl of sample to each of the rest of the wells. (Do not blank well A1 when you plan to read the absorbance using a reference wavelength)
2. Add 50 μl of enzyme conjugate to each well except the blank well.
3. Shake on a plate shaker for 30 seconds to completely mix the liquid within the wells.
4. Cover the plate with a lid and incubate at 37 ℃ for 30 minutes.
5. Add 350 μl of wash solution, decant (tap and blot) or aspirate. Repeat 5 additional times for a total of 6 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
6. Add 50 μl of substrate A, then 50 μl of substrate B to each well, including the blank well.
7. Gently mix for 15 seconds and incubate at 37 ℃ in the dark for 10 minutes without shaking.
8. Add 50 μl of stop solution to each well, including the blank well and mix gently.
9. Read the absorbance within 20 minutes at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfections) in a microplate reader. Alternatively, the actual absorbance can be obtained by subtracting the absorbance of each well at 450 nm with the absorbance of the blank well at 450 nm.

Procedural Flow Chart



Measurement Results

Each plate must be considered separately when calculating and interpreting results of the assay.

1. Negative Control

Calculate the mean absorbance of the replicates of the negative control.

2. Cut-off Value

The cut-off value is 0.3 times the mean of the negative control replicates.

3. Example

Negative Control absorbance:	well 1 = 3.564, well 2 = 3.780, well 3 = 3.812
Mean Negative Control	= (3.564 + 3.780+ 3.812)/3 = 3.719
Cut-off Value	=0.3 x 3.719= 1.116

Control Procedure

The recommended control requirement for this assay is using positive and negative controls to verify assay performance. The result is valid if the following criteria for the controls are both met:

1. Negative Control

Mean absorbance of negative control is higher than 0.8.

2. Positive Control

Mean absorbance of positive control is lower than 0.1.

Interpretation of Results

1. Nonreactive

Samples giving an absorbance equal to or greater than the cut-off value are considered nonreactive.

2. Reactive

Samples giving an absorbance less than the cut-off value are considered reactive. Unless local procedures state otherwise, such samples must be retested in duplicate using the original source. Samples that are reactive in at least one of the duplicate retests are considered repeatedly reactive in this assay and are presumed to contain antibodies to HBc. Such samples must be further investigated and the results from this assay considered with any other clinical and/or assay information. Samples which are non-reactive in both wells on retest must be considered nonreactive in this assay.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.

Performance Characteristics

1. Measurement Precision

This assay is designed to have a within-run precision of <10%. 2 human plasma based panel members (1 and 2) were assayed, using 1 batch of reagents, in replicates of 20. Data from this study are summarized in the following table.

Panel Member	Batch	n	Mean	Within-run Precision SD	%CV
1	1	20	1.060	0.065	6.1
2	1	20	0.423	0.034	8.0

This assay is designed to have a between-batch precision of <15%. 2 human plasma based panel members (1 and 2) were assayed, in replicates of 8, using 3 batches of reagents. Data from this study are summarized in the following table.

Panel Member	n	Mean	Between-batch Precision SD	%CV
1	24	1.266	0.136	10.7
2	24	0.540	0.060	11.1

2. Sensitivity

The diagnostic sensitivity of the product was determined by testing a panel of 100 positive samples, all samples resulted positive, therefore the diagnostic sensitivity was 100%.

Samples	Tested Samples	Positive Samples
Anti-HBc Positive	100	100

3. Specificity

The diagnostic specificity was determined by testing a panel of 2100 samples, in parallel with a test already available on the market. The results obtained show a diagnostic specificity of 99.80%.

		This Assay		
		Positive	Negative	Total
Reference Test	Positive	100	0	100
	Negative	4	1996	2000
	Total	104	1996	2100

Literature References

1. Kimura T, Rokuhara A, Matsumoto A, et al. New enzyme immunoassay for detection of hepatitis B virus core antigen (HBcAg) and relation between levels of HBcAg and HBV DNA. *J. Clin. Microbiol.* 2003;41(5):1901-1906.

2. Cao T, Meuleman P, Desombere I, Sällberg M, Leroux-Roels G. In vivo inhibition of anti-hepatitis B virus core antigen (HBcAg) immunoglobulin G production by HBcAg-specific CD4(+) Th1-type T-cell clones in a hu-PBL-NOD/SCID mouse model. *J. Virol.* 2001;75(23):11449-11456.

3. Weber B, Melchior W, Gehrke R, et al. Hepatitis B virus markers in anti-HBc only positive individuals. *J. Med. Virol.* 2001;64(3):312-319.

4. Bodsworth NJ, Cooper DA, Donovan B. The influence of human immunodeficiency virus type 1 infection on the development of the hepatitis B virus carrier state. *J. Infect. Dis.* 1991;163(5):1138-1140.

5. Hollinger FB. Hepatitis B virus infection and transfusion medicine: science and the occult. *Transfusion*. 2008;48(5):1001-1026.

6. Araujo NM, Waizbort R, Kay A. Hepatitis B virus infection from an evolutionary point of view: how viral, host, and environmental factors shape genotypes and subgenotypes. *Infect. Genet. Evol.* 2011;11(6):1199-1207.

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium