Prestige Diagnostics
Clinical Diagnostics & Point of Care Products

Anti HBc ELISA

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CAT NO	DESCRIPTION	PACK SIZE
EIAHBC1	Anti HBc ELISA	96 Tests

Intended Use:

Anti HBc ELISA is an enzyme linked immunosorbent assay for qualitative detection of antibodies to hepatitis B virus core antigen (anti HBc) in human serum or plasma. It is intended for use in clinical laboratories for diagnosis and management of patients with infection by hepatitis B virus. This reagent is for *In vitro* Diagnostic use only.

Summary and Principle:

Hepatitis B virus (HBV) is an enveloped, double-stranded DNA virus belonging to the Hepadnaviridae family and is recognized as the major cause of blood transmitted hepatitis together with hepatitis C virus (HCV). Infection with HBV induces a spectrum of clinical manifestations ranging from mild, inapparent disease to fulminant hepatitis, severe chronic liver diseases, which in some cases can lead to cirrhosis and carcinoma of the liver. Classification of a hepatitis B infection requires the identification of several serological markers expressed during three phases (incubation, acute and convalescent) of the infection. Now several diagnostic tests are used for screening, clinical diagnosis and management of the disease.

Hepatitis core antigen is a major component of the viral structure. It is released on disaggregation of the core particles. The antigen contains at least one immunological determinant. Antibodies to HBcAg appear shortly after the appearance of HBsAg and persist for life in persons who have recovered from Hepatitis B infection.

Anti-HBc ELISA is based on a one step incubation, competitive ELISA method. Anti-HBc antibody if present in the sample, competes with monoclonal anti-HBc conjugated to horseradish peroxidase (Conjugate) for a fixed amount of purified Hepatitis B core antigen pre-coated on the wells. With no anti-HBc in the sample, only conjugated anti-HBc will be bound with the antigen on the wells, giving maximum signal. Substrate A and B solutions are added into the wells and during incubation, the colourless chromogen is hydrolysed by the bound HRP-Conjugate to a blue-coloured product. The blue colour turns yellow after stopping the reaction with sulfuric acid. No or low colour development is indicative of the presence of antibodies to Hepatitis B core antigen in the sample.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with purified HBcAg. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable for 1 month at 2-8°C.
Negative Control	1x1ml	Pale Yellow liquid in a vial with green screw cap. Protein stabilized buffer tested non-reactive for anti-HBc. Ready to use. Once open stable for 1 month at 2-8°C.
Positive Control	1x1ml	Red coloured liquid in a vial with red screw cap. Purified anti-HBc diluted in protein stabilized buffer. Ready to use. Once open stable for 1 month at 2-8°C.
Conjugate	1x6ml	Red coloured liquid in a white vial with screw cap. Horseradish peroxidise conjugated anti-HBc. Once open, stable for one month at 2-8°C.
Wash Buffer (20X)	1x20ml	PBS at pH 7.4. 20X concentrate. Once open, stable for one month at 2-8°C. The concentrate must be diluted 1 to 20 with distilled water before use. Once diluted, stable for one week at room temperature, or two weeks when stored at 2-8°C.
Substrate A	1x6ml	Urea peroxide solution. Ready to use. Once open, stable for one month at 2-8°C.
Substrate B	1x6ml	TMB Solution. Ready to use. Once open, stable for one month at 2-8°C.
Stop Solution	1x6ml	Diluted Sulfuric acid solution (0.5M) Ready to use. Once open, stable for 1 month at 2-8°C.

Plastic Sealable bag, IFU and plate cover.

Materials provided but not required:

Distilled water or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, dispensing systems, disposable pipette tips, absorbent tissue or clean towel, dry bath incubator or water bath, plate reader, single wavelength 450nm or dual wavelength 450/630nm and microwell aspiration systems.

Specimen Collection:

1. No special patient preparation is required. Collect the specimen in accordance with normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venepuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particulate matters in the specimens should be removed by centrifugation at 3000 RPM for 20 minutes at room temperature or by filtration.
2. Plasma specimens collected into EDTA, sodium citrate or heparin can be tested, but highly lipaemic, icteric or haemolytic specimens should not be used as they give false results in the assay. Do not heat inactivate specimens. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.
3. The AMS Anti HBc ELISA assay is used only for testing serum or plasma samples. Do not use for testing cadaver samples, saliva, urine or other body fluids or pooled (mixed) blood.
4. Transportation and Storage: store specimens at 2-8°C. Specimens not required for assaying within 7 days should be stored at -20oC or lower. Multiple free thaw cycles should be avoided. For shipment, samples should be packaged and labelled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination.

Precautions and Safety:

The ELISA assays are time and temperature sensitive. To avoid incorrect results, strictly follow the test procedure.

1. **Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.**
2. **Make sure that all reagents are within the expiry indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.**
3. **Important: Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.**
4. **Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.**
5. **Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.**
6. **Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.**
7. **Avoid assay steps long time interruptions. Assure same working conditions for all wells.**
8. **Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.**
9. **Assure that the incubation temperature is 37°C inside the incubator.**
10. **When adding specimens, do not touch the well's bottom with the pipette tip.**
11. **When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.**
12. **The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.**
13. **If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.**
14. **All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.**
15. **WARNING: Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.**
16. **Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.**
17. **Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.**
18. **The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121oC or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should never be autoclaved. MSDS available upon request.**
19. **Some reagents may cause toxicity, irritation, burns or have carcinogenic effects as raw materials. Contact with skin and the mucosa should be avoided but not limited to the following reagents: stop solution, chromogen reagents and the wash buffer.**
20. **The Stop Solution contains sulfuric acid. Use it with appropriate care. Wipe up spills, immediately and wash with water if comes into contact with the skin or the eyes.**
21. **Proclin 300 is used as preservative and can cause sensation of the skin. Wipe up spills immediately or wash with water if comes into contact with skin or eyes.**
22. **Indications of instability or deterioration of the reagents: The values of positive and negative controls which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and or operator or equipment errors. In such cases, the results should be considered as invalid and the samples must be retested. In case of consistently erroneous results and proven deterioration or instability of the reagents, immediately discard the reagents in use and use a new kit. Contact the local AMS representative.**

Procedure:

Reagent preparation:

Allow the reagents to reach room temperature (18-30°C). Check the wash buffer concentration for the presence of salt crystals. If crystals have formed, re-solubilize by warming at 37°C, until crystals dissolve. Dilute the wash buffer (20X) as indicated in the instructions for washing. Use distilled or deionized water and clean vessels to dilute the buffer. All other reagents are ready to use as supplied.

STEP 1

Preparation: Reserve 2 wells for Negative Control, two wells for Positive Control and one Blank (A1 – neither samples nor HRP conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

STEP 2

Addition of the sample and Conjugate: Add 50 µl of Positive control, Negative Control and specimen to their respective wells except the blank. Note: Use a separate disposable tip for each specimen and standard to avoid cross-contamination. Add 50 µl of Conjugate to each well except the Blank. Mix by tapping the plate gently.

STEP 3

Incubation: Cover the plate with the plate cover and incubate for 30 minutes at 37°C.

STEP 4

Washing: At the end of the incubation period, remove and discard the plate cover and the contents of the microwells. Wash each well 5 times with diluted washing buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 5

Addition of the Substrate: Add 50 µl of Substrate A and 50 µl of Substrate B to each well including the blank. Incubate the plate at 37°C for 10 minutes in the dark.

STEP 6

Stopping the Reaction: Add 50 µl of the Stop solution to each well and mix gently. Intensive yellow colour develops in the Negative Control and Anti-HBc negative sample wells (previously blue).

STEP 7

Measurement: Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the cut off value and evaluate the results. (Note: Absorbances must be read within 10 minutes of adding the stop solution).

Instructions for Washing:

- To remove any effect of washing on false reactions, a 5 automatic wash cycle is required with 350-400ul of diluted wash buffer used per well per wash. This helps in avoiding false positive reactions and a high background.
- To avoid cross-contamination of the plate with specimen or HRP conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
- Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of wash buffer is dispensed each time into the wells.
- In case of manual washing, we suggest carrying out 5 washing cycles, dispensing 350-400ul/well and aspirating the liquid 5 times. If poor results are observed with high background, increase washing cycles to soak time per well.
- Treat the liquid aspirated after the reaction from the wells with Sodium hypochlorite (at a concentration of 2.5%) for 24 hours before they are disposed off in the appropriate way.
- The concentrated wash buffer should be diluted 1/20 before use. If less than a whole plate is used, prepare the proportional volume of solution.

Calculation of results:

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (Abs) value to the Cut-off value (CO) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well Abs value from the print report values of specimens and controls. If the reading is based on dual filter plate reader, do not subtract the Blank well Abs value from the print report values of specimens and controls.

Calculation of the Cut-off value (CO) = Pabs × 0.6 + Nabs 0.4

(Pabs = the mean absorbance value for the 2 positive controls

Nabs = the mean absorbance value for the 2 negative controls).

Quality control (assay validation): The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

- The Abs value of the Blank well, which contains only Substrate and Stop solution, is < 0.080 at 450 nm.
- The Abs values of the Positive control must be ≤ 0.100 at 450/630nm or at 450nm after blanking.
- The Abs values of the Negative control must be ≥ 0.800 at 450/630nm or at 450nm after blanking.

If one of the Negative control Abs values does not meet the Quality Control criteria, it is to be discarded, and the other value used to calculate CO. If both Negative control Abs values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Example:

1. Quality Control

Blank well Abs value: A1 = 0.025 at 450nm (Note: blanking is required only when reading with single filter at 450nm)

Well No.:	**B1**	**C1**
Negative control Abs values after blanking:	1.750	1.735
Well No.:	**E1**	**F1**
Positive control Abs values after blanking:	0.012	0.009

All control values are within the stated quality control range

2. Calculation of Nabs: = $\frac{(1.750+1.735)}{2}$ = 1.743

3. Calculation of Pabs: = $\frac{(0.012+0.009)}{2}$ = 0.011

3. Calculation of the Cut-off: (CO) = 0.011 × 0.6 + 1.743 x 0.4 = 0.704

Interpretation of results:

Negative Results (Abs / CO > 1): Specimens giving Abs value greater than the Cut-off value are negative for this assay, which indicates that no anti-HBc have been detected with this anti-HBc ELISA. This result should not be used alone to establish the infection state.

Positive Results (Abs / CO ≤ 1): Specimens giving Abs value less than or equal to the Cut-off value are considered initially reactive, which indicates that anti-HBc have been detected with this anti-HBc ELISA. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for anti-HBc. A positive result with anti-HBc detection is an indication of acute HBV infection. Determination of anti-HBc is useful method for screening of blood donors and in serological monitoring during follow-up of chronic HBV carriers. However, any positive result should not be used alone to establish the infection state.

Borderline (Abs / C.O. = 0.9-1.1): Specimens with Abs value to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results. Repeatedly reactive samples could be considered positive for anti-HBc.

- If after retesting of the initially reactive samples, both wells are negative results (Abs/C.O.>1.1), these samples should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step.

- If after retesting in duplicates, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for anti-HBc.
- After retesting in duplicates, samples with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone sample, or uninterpretable for the time of testing.

Performance Characteristics:

Analytical Endpoint Sensitivity: The sensitivity of the assay has been calculated by means of the reference standards.

The clinical specificity of this assay has been determined using samples obtained from 1683 healthy blood donors and 145 undiagnosed hospitalized patients.

The clinical sensitivity of this anti-HBc ELISA kit has been calculated using samples obtained from 975 hepatitis B patients with well-characterized clinical history based upon reference assays for detection of HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc. This panel included samples from acute, chronic and recovered hepatitis B patients. Licensed anti-HBc ELISA test was used as a confirmatory assay. The evaluation results are given below. Results obtained in individual laboratories may differ.

Specificity	Number of Samples	-	+	Confirmed Positive	Specificity	False Positive
Blood donors	1683	566	1117	1115	99.64%	2
Patients	145	80	65	65	100%	0
Total	1828	646	1182	1180	99.82%	2

Sensitivity	Number of Samples	-	+	Confirmed Positive	Sensitivity	False Negative
Acute	429	11	417	418	99.76%	1
Chronic	105	0	105	105	100%	0
Recovery	441	5	436	436	100%	0
Total	975	16	958	959	99.82%	1

Analytical Specificity:
No cross reactivity observed with samples from patients infected with HAV, HCV HIV, CMV, and TP.
No interference from rheumatoid factors up to 2000U/ml observed during clinical testing.
The assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein.
Frozen specimens have been tested to check for interferences due to collection and storage.

Limitations:

1. Positive results must be confirmed with another available method and interpreted in conjunction with the other patient clinical information.
2. Antibodies may be undetectable during the early stage of the disease and in some immunosuppressed individuals. In very rare cases some HBV mutants or subtypes can remain undetectable. Therefore, negative results obtained with this anti-HBc ELISA are only indication that the sample does not contain detectable level of anti-HBc.
3. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step
4. The most common assay mistakes are: using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.
5. This kit is intended only for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
6. This kit is a qualitative assay and the results cannot be used to measure antibody concentration.

References:

1. Hansson, B.G. (1977). Persistence of Serum Antibody to Hepatitis B Core Antigen. J. Clin. Microbiol. 6, 209
2. Hoofnagle, J.H., Gerety, R.J. and Barker, L.F. (1973). Antibody to Hepatitis B Virus Core in man. Lancet, 869
3. Hoofnagle, J.H., Gerety, R.J., Ni, L.Y. and Barker, L.F. (1974)
4. Antibody to Hepatitis B Core Antigen. N. Engl. J. Med., 290, 1336
5. Mushahwar, I.K.,Dienstag,J .L.,Polesky,H.F et al (1981) Interpretation of Various Serological Profiles of Hepatitis B Virus Infection.Am J. Clin Pathol, 76, 773

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

Prestige Diagnostics U.K. Ltd 40 Ballymena Business Centre, Galgorm, Co. Antrim, BT42 1FL, United Kingdom. Tel: +44 (0) 28 2564 2100

www.prestigediagnostics.co.uk info@prestigediagnostics.co.uk

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