Prestige Diagnostics
Clinical Diagnostics & Point of Care Products

HBeAg Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIAHE01	HBeAg Elisa	96 Tests

Intended Use:

HBeAg Elisa is an enzyme linked immunusorbent Elisa assay for the qualitative detection of Hepatitis B virus envelope antigen in human serum and plasma. 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 B "e" antigen is a virus protein to be intimately associated with hepatitis B virus replication, indicating high degree of infectivity. HBeAg appears shortly after HBsAg and is detectable for few days to several weeks. During treatment and recovery, the titer of HBeAg declines and is replaced by the corresponding antibody (anti-HBe). In chronic hepatitis B infections, elevated levels of HBeAg can be detected for years, which is a marker for large quantity of virus. In some chronic HBsAg positive patients, HBeAg is undetectable due to HBV mutations suggesting for low level of viral replication. If HBeAg is considered a specific marker of infectivity, the presence of anti-HBeAg antibody in blood is recognized to be a clinical sign of recovery from the infection.

HBeAg ELISA uses "sandwich" ELISA method in which, polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBeAg. Patient's serum or plasma sample is added to the microwell together with a second monoclonal antibody conjugated to horseradish peroxidase (HRP-Conjugate). During incubation, the specific immunocomplex formed in case of presence of HBeAg in the sample is captured on the solid phase. After washing to remove sample and unbound HRP-Conjugate, Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added into the wells. In presence of the antibody-antigen-antibody(HRP) "sandwich" complex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antigen captured in the wells, and to the sample respectively. Wells containing samples negative for HBeAg remain colorless.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with monoclonal antibodies reactive to HBeAg. 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	Yellowish liquid filled in a vial with green screw cap. Protein stabilized buffer tested non-reactive for HBeAg. Ready to use. Once open stable for 1 month at 2-8°C.
Positive Control	1x1ml	Red coloured liquid filled in a vial with red screw cap. Recombinant non infective HBeAg diluted in protein stabilized buffer. Ready to use. Once open stable for 1 month at 2-8°C.
HRP-Conjugate	1x6.5ml	Red coloured vial in a white vial with screw cap. Horseradish peroxidise conjugated anti-HBe antibodies. Once open, stable for one month at 2-8°C.
Wash Buffer (20X)	1x30ml	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.
Chromogen A	1x7ml	Urea peroxide solution. Ready to use. Once open, stable for one month at 2-8°C.
Chromogen B	1x7ml	TMB Solution. Ready to use. Once open, stable for one month at 2-8°C.
Stop Solution	1x8ml	Diluted Sulfuric acid solution (0.5M) Ready to use. Once open, stable for 1 month at 2-8°C.

Plastic Sealable bag, IFU and Cardboard plate covers.

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 Prestige Anti HBc Elisa assay is used only for testing individual 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 and do not modify them.

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 validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. CAUTION - CRITICAL STEP: 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 Prestige 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: Mark 3 wells as Negative control (B1,C1,D1), two wells as Positive control (E1,F1) 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 & HRP Conjugate: Add 50ul of Positive control, Negative Control and specimen into their respective wells except the

blank. Note: Use a separate disposable tip for each specimen and standard to avoid cross-contamination. Add 50ul of HRP conjugate into each well except the Blank. Mix by tapping the plate gently.

STEP 3

Incubation: Cover the plate with the plate cover and incubate for 60 minutes at 37°C.

STEP 4

Washing: At the end of the incubation period, remove and discard the plate cover. 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 chromogens: Add 50ul of Chromogen A and 50ul of Chromogen B into each well including the blank. Incubate the plate at 37°C for 15 minutes avoiding light. The enzymatic reaction between the chromogen solutions and the HRP conjugate produces blue colour in Positive control and HBeAg Positive samples.

STEP 6

Stopping the Reaction: Add 50ul of the Stop solution into each well and mix gently. Intensive yellow colour develops in the positive control and HBeAg positive sample wells.

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 washing on false positive 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 to carry 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 (A) value to the Cut-off value (C.O.) 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 A value from the print report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well A value from the print report values of specimens and controls.

Calculation of the Cut-off value (C.O.) = Nc × 2.1
(**Nc** = the mean absorbance value for three negative controls).
Important: If the mean A value of the negative controls is lower than 0.05, take it as 0.05.

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

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded and the mean value calculated again using the remaining two values. If more than one Negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Example:

1. Quality Control
Blank well A value: A1= 0.025 at 450nm (Note: blanking is required only when reading with single filter at 450nm)

Well No.:	**B1**	**C1**	**D1**
Negative control A values after blanking:	0.020	0.012	0.016
Well No.:	**E1**	**F1**	
Positive control A values after blanking:	2.421	2.369	

All control values are within the stated quality control range
2. Calculation of Nc: = $\frac{(0.020+0.012+0.016)}{3}$ = 0.016 (Nc is lower than 0.05, so take it as 0.05)
3. Calculation of the Cut-off: (C.O.) = 0.05 × 2.1 = 0.105

Interpretation of results:

- **Negative Results** (A / C.O. < 1): Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no HBeAg has been detected with this HBeAg ELISA. This result should not be used alone to establish the infection state.
- **Positive Results** (A / C.O. ≥ 1): Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that HBeAg has probably been detected using this HBeAg ELISA. All initially reactive specimens should be retested in duplicates using this HBeAg ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for HBeAg with this HBeAg ELISA. However, any positive result should not be used alone to establish the infection state.
- **Borderline** (A / C.O. = 0.9-1.1): Specimens with absorbance 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 can be considered positive for HBeAg.
- **Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.**

- If, after retesting of the initially reactive samples, both wells are negative results (A/C.O.<0.9), 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 HBeAg.
- 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:

Clinical Specificity: This clinical specificity of this kit has been determinate by a panel of samples obtained from 4360 healthy blood donors and 150 undiagnosed hospitalized patients. The repeatedly reactive samples and samples confirmed positive with the reference test were not included in the calculation of specificity.

Clinical Sensitivity: The clinical sensitivity of this HBeAg ELISA was calculated by a panel of samples obtained from 813 hepatitis B patients with well-characterized clinical history based upon reference assays for detection of HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc. Licensed HBeAg ELISA was used as a confirmatory assay. The evaluation results are given below.

Specificity	Number of Sample	-	+	Confirmed Positive	Specificity	False Positive
Donors	4360	4346	14	9	99.86%	5
Patients	150	132	18	18	100%	0
Total	4510	4478	32	27	99.93%	5

Sensitivity	Number of Sample	-	+	Confirmed positive	Sensitivity	False Negatives
Acute	378	172	206	206	100%	0
Chronic	347	162	185	185	100%	0
Recovery	88	63	25	25	100%	0
Total	813	397	416	416	100%	0

Analytical Specificity:
No cross reactivity was observed with samples from patients infected with HAV, HCV, HIV, CMV, and TP.
No interference from rheumatoid factors up to 2000U/ml and no high dose hook effect up to HBeAg concentrations of 150000NCU were observed.
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 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 HBeAg ELISA are only indication that the sample does not contain detectable level of HBeAg.
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. The prevalence of the marker will affect the assay's predictive values.
6. 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.
7. This kit is a qualitative assay and the results cannot be used to measure antibody concentration.

References:

1. Dienstag J.L. Hepatitis A virus: identification, characterization and epidemiological investigations. Progress in liver disease VI, Popper E., Schaffner F. (Eds), pp. 343-370, New York, Gruner and Stratton, 1979.
2. Duermeyer W., Van der Veen J., Koster B.ELISA in hepatitis A. Lancet. i : 823-824, 1978.
3. J.V. PARRY, (1981). Hepatitis A infection: guidelines for development of satisfactory assays for laboratory diagnosis.The Institute of Medical Laboratory Sciences 38, 303-311.
4. Lindberg J., Frosner G., Hansson B.G. et al. Serologic markers of hepatitis A and B in chronic active hepatitis. Scandinavian Journal of Gastroenterology,13:525-527, 1978.

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

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