Hepatitis B virus e Antigen (HbeAg) ELISA Kit

Catalog Number KA3288
96 assays
Version: 02

Intended for research use only
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Introduction

Intended Use

This enzyme-linked immunosorbent assay (ELISA) kit is for the qualitative detection of hepatitis B virus e antigen (HBeAg) in human serum or plasma.

Background

Hepatitis is recognized as the major cause of blood transmitted hepatitis together with the hepatitis C virus (HCV). The Hepatitis B virus (HBV) is an enveloped, double-stranded DNA virus belonging to the Hepadnaviridae family. Infection with HBV induces a spectrum of clinical symptoms ranging from mild, inapparent disease to fulminant hepatitis, severe chronic liver disease, 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. Various diagnostic tests are used for screening, clinical diagnosis and treatment of the disease.

The Hepatitis B “e” antigen, is a virus protein that is closely associated with the hepatitis B virus replication, indicating a high degree of infectivity. HBeAg normally appears shortly after HBsAg, and is detectable from a few days to several weeks later. 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 sign for a heavy quantity of the virus. In some chronic HBsAg positive patients, HBeAg is undetectable due to HBV mutations suggesting low levels of viral replication. The anti-HBeAg antibody appearance in the blood is recognized to be a clinical sign of recovery from the infection, respectively, if HBeAg is considered the specific marker of infectivity in that patient.

Principle of the Assay

This Hepatitis B kit has a “sandwich” ELISA method in which, polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBeAg. Human serum or plasma specimen is added to the microwell together with a second monoclonal antibody conjugated to horseradish peroxidase (HRP-Conjugate). The specific immunocomplex formed in case of presence of HBeAg in the sample is captured on the solid phase, during incubation. After the washing step to remove sample and unbound HRP-Conjugate, Chromagen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added into the wells. In presence of the antibody-antigen-antibody(HRP) “sandwich” complex, the Chromagens, which are colorless, are hydrolyzed by the bound HRP conjugate to a blue-colored product. After stopping the reaction with sulfuric acid, the blue color turns yellow. The amount of color intensity can be measured with an instrument. The intensity is proportional to the amount of antigen captured in the wells, and to the sample, respectively. Negative sample wells for HBeAg remain colorless.
Assay principle scheme:
Double antibody sandwich ELISA

\[ \text{Ab(p)} + \text{Ag(s)} + \text{(Ab)ENZ} \rightarrow [\text{Ab(p)} - \text{Ag(s)} - \text{(Ab)ENZ}] \rightarrow \text{blue} \rightarrow \text{yellow color ( + )} \]

\[ \text{Ab(p)} + \ \text{(Ab)ENZ} \rightarrow [\text{Ab(p)} \rightarrow \text{no color ( - )} \]

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Immobilize Complex</th>
<th>Coloring</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>60min.</td>
<td></td>
<td>15min.</td>
<td></td>
</tr>
</tbody>
</table>

\text{Ab(p): pre-coated anti–HBe antibodies}
\text{Ag(s): HBeAg antigens in sample}
\text{(Ab)ENZ: HRP conjugated anti-HBe antibodies}
**General Information**

**Materials Supplied**

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwell Plate: Blank microwell strips fixed on white strip holder. The plate is sealed in aluminium pouch with desiccant. Each well contains monoclonal antibodies reactive to HBeAg. The microwell strips can be broken to be used separately. Place unused wells or strips in the plastic sealable storage bag together with desiccant and return to 2-8°C.</td>
<td>96(8x12) wells</td>
</tr>
<tr>
<td>Negative Control: Yellowish liquid filled in a vial with green screw cap. Protein-stabilized buffer tested non-reactive for HBeAg. Preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8°C.</td>
<td>1 mL</td>
</tr>
<tr>
<td>Positive Control: Red-colored liquid filled in a vial with red screw cap. Recombinant, non-infective HBeAg diluted in protein-stabilized buffer containing preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8°C.</td>
<td>1 mL</td>
</tr>
<tr>
<td>HRP-Conjugate Reagent: Red-colored liquid filled in a white vial with red screw cap. Horseradish peroxidase-conjugated anti-HBe antibodies. Ready to use as supplied. Once open, stable for one month at 2-8°C.</td>
<td>6.5 mL</td>
</tr>
<tr>
<td>Stock Wash Buffer: Colorless liquid filled in a clear bottle with white screw cap. PH 7.4 20 × PBS (Containing Tween-20 as a detergent). *Dilute Before Use-The concentrate must be diluted 1 to 20 with distilled/deionized water before use. Once diluted, stable for one week at room temperature or for two weeks at 2-8°C.</td>
<td>30 mL</td>
</tr>
<tr>
<td>Chromogen Solution A: Colorless liquid filled in a white vial with green screw cap. Urea peroxide solution. Ready to use as supplied. Once open, stable for one month at 2-8°C.</td>
<td>7 mL</td>
</tr>
<tr>
<td>Chromogen Solution B: Colorless liquid filled in a Brown vial with brown screw cap. TMB solution (Tetramethyl benzidine dissolved in citric acid). Ready to use as supplied. Once open, stable for one month at 2-8°C</td>
<td>7 mL</td>
</tr>
<tr>
<td>Stop Solution: Colorless liquid in a white vial. Diluted sulfuric acid solution (2.0M H₂SO₄) Ready to use as supplied.</td>
<td>7 mL</td>
</tr>
<tr>
<td>Plastic Sealable Bag: For enclosing the strips not in use.</td>
<td>1 unit</td>
</tr>
<tr>
<td>Cardboard Plate Cover: To cover the plates during incubation and prevent evaporation or contamination of the wells.</td>
<td>1 sheet</td>
</tr>
</tbody>
</table>
Storage Instruction

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8 °C; do not freeze. To assure maximum performance of this HBeAg ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

Materials Required but Not Supplied

✓ Freshly distilled or deionized water.
✓ Disposable gloves and timer.
✓ Appropriate waste containers for potentially contaminated materials.
✓ Disposable V-shaped troughs.
✓ Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
✓ Absorbent tissue or clean towel.
✓ Dry incubator or water bath, 37±0.5°C.
✓ Microshaker for dissolving and mixing conjugate with samples.
✓ Microwell plate reader, single wavelength 450 nm or dual wavelength 450 nm and 630 nm.
✓ Microwell aspiration/wash system.

Precautions for Use

✓ Precautions and Safety
  • This kit is intended FOR RESEARCH USE ONLY
  • FOR PROFESSIONAL USE ONLY
  • The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.
  • Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
  • Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
  • Caution - Critical Step: Allow the reagents and samples to stabilize at room temperature (18-30°C) before use. Shake reagent gently before, and return to 2-8°C immediately after use.
  • Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
  • Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
  • When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
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.

Avoid assay steps long time interruptions. Assure same working conditions for all wells.

Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.

The use of automatic pipettes is recommended.

Assure that the incubation temperature is 37°C inside the incubator.

When adding samples, avoid touching the well’s bottom with the pipette tip.

When reading the results with a plate reader, it is recommended to determine the absorbance at 450 nm or at 450 nm with reference at 630nm.

All specimens from human origin should be considered as potentially infectious.

Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV ½, 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. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.

Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.

The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1 hour at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps for disposal.

The Stop solution (2M H₂SO₄) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.

The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such substances.

If using fully automated microplate processing system, 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.

Limitations of the Procedure

Non-repeatable positive result may occur due to the general biological and biochemical characteristics of ELISA method. The kit is designed to achieve performance characteristics of very high sensitivity and specificity. However, in very rare cases, some HBV mutants or subtypes could remain undetectable. Antibodies may also be undetectable during the early stages of the disease and in some immunosuppressed individuals.
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.

Any positive result must be interpreted in conjunction with other laboratory testing results.

Common sources for mistakes: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.

The prevalence of the marker will affect the assay’s predictive values.

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.

This is a qualitative assay and the results cannot be used to measure antigens concentrations.
Assay Protocol

Reagent Preparation

Allow the reagents and samples to reach room temperature (18-30°C) for at least 15-30 minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37 until crystals dissolve. Dilute the stock Wash Buffer 1 to 20 with distilled or deionized water. Use only clean vessels to dilute the buffer.

Sample Preparation

✓ Sample Collection: Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.

✓ Transportation and Storage: Store samples at 2-8°C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transport of clinical samples and ethological agents.

Assay Procedure

1. Numbering Wells: Set the strips needed in strip-holder and number sufficient number of wells including three Negative controls (e.g. B1, C1, D1), two Positive controls (e.g. E1, F1) and one Blank (e.g. 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.

2. Adding Samples and HRP-Conjugate: Add 50 µl of Positive control, Negative control, and specimen into their respective wells. Note: Use a separate disposal pipette tip for each specimen, Negative and Positive Controls as to avoid cross-contamination. Add 50 µl HRP-Conjugate to each well except into the Blank and mix by tapping the plate gently.

3. Incubating: Cover the plate with the plate cover and incubate for 60 minutes at 37°C. It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.
4. Washing: At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash buffer. Each time, allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remainders.

5. Coloring: Dispense 50 µl of Chromogen A and 50 µl Chromogen B solution into each well including the Blank and mix by tapping the plate gently. Incubate the plate at 37°C for 15 minutes avoiding light. The enzymatic reaction between the Chromogen A/B solutions and the HRP-Conjugate produces blue color in Positive control and HBeAg positive sample wells.

6. Stopping Reaction: Using a multichannel pipette or manually, add 50 µl Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and HBeAg positive sample wells.

7. Measuring the Absorbance: Calibrate the plate reader with the Blank well and read the absorbance at 450 nm. If a dual filter instrument is used, set the reference wavelength at 630 nm. Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 5 minutes after stopping the reaction).

✓ Validity

Please do not use this kit beyond the expiry date indicated on the kit box and reagent labels.

✓ Special instructions for washing

- A good washing procedure is essential to obtain correct and precise analytical data.

- It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles with dispensing of 350-400 µl/well, are sufficient to avoid false positive reactions and high background (all wells turn yellow).

- To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the content of the wells, but allow the plate washer to aspirate it automatically.

- Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer’s 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 perform at least 5 cycles, dispensing 350-400 µl/well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.

- In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution (final concentration of 2.5%) for 24 hours, before liquids are disposed in an appropriate way.

- The concentrated Washing solution should be diluted 1 to 20 before use. For one plate, mix 30 ml of the concentrate with 570 ml of water for a final volume of 600 ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.
Data Analysis

Calculation of Results

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample’s optical density (OD) 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 OD value from the print report values of samples and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

✓ Calculation of Cut-off value (C.O.) = *Nc × 2.1
*Nc = the mean absorbance value for three negative controls.

Important: If the mean OD value of the negative control is lower than 0.05, take it as 0.05.

Example:

1. Calculation of Nc:

<table>
<thead>
<tr>
<th>Well No</th>
<th>B1</th>
<th>C1</th>
<th>D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative controls OD value</td>
<td>0.02</td>
<td>0.012</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Nc=0.016 (Nc is lower than 0.05 so take it as 0.05)

Calculation of Cut-off value: (C.O.) = 0.05 × 2.1 = 0.105

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

✓ Quality control range

The test results are valid if the Quality Control criteria are verified. It is advisable that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the sample being analyzed.

- The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.
- The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm or at 450nm after blanking.
- The OD value of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.
Interpretations of the results

(S = the individual absorbance (OD) of each specimen)

- Negative Results (S/C.O. <1): Samples giving absorbance less than the Cut-off value are negative for this assay, which indicates that no hepatitis B virus e antigen has been detected with this HBeAg ELISA kit. This result should not be used alone to establish the infection state.

- Positive Results (S/C.O. ≥ 1): Samples giving an absorbance greater than, or equal to the Cut-off value are initially reactive, which indicates that HBV e antigen has probably been detected using this HBeAg ELISA kit. Any initially reactive samples should be retested in duplicates. Repeatedly reactive samples could be considered positive for HBeAg. This result should not be used alone to establish the infection state.

- Borderline (S/CO=0.9-1.1): Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline samples and retesting is recommended. Repeatedly reactive samples can be considered positive for HBeAg.

Performance Characteristics

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

- Sensitivity: The sensitivity of this HBeAg ELISA kit was calculated by a panel of samples obtained from 813 hepatitis B individuals 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. Results obtained in individual laboratories may differ.

- Analytical Specificity: No cross reactivity was observed with samples from individuals with HAV, HCV, HIV, CMV, and TP. No interference from rheumatoid factors up to 2000 U/ml and no high dose hook effect up to HBeAg concentrations of 150000 NCU 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.

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Samples</th>
<th>-</th>
<th>+</th>
<th>Confirmed positive</th>
<th>Specificity</th>
<th>False positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donors</td>
<td>4360</td>
<td>4346</td>
<td>14</td>
<td>9</td>
<td>99.86%</td>
<td>5</td>
</tr>
<tr>
<td>Samples</td>
<td>150</td>
<td>132</td>
<td>18</td>
<td>18</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>4510</td>
<td>4478</td>
<td>32</td>
<td>32</td>
<td>99.93%</td>
<td>5</td>
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<tr>
<td>Sensitivity</td>
<td>Samples</td>
<td>-</td>
<td>+</td>
<td>Confirmed positive</td>
<td>Specificity</td>
<td>False positive</td>
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<tr>
<td>Acute</td>
<td>378</td>
<td>172</td>
<td>206</td>
<td>206</td>
<td>100%</td>
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<tr>
<td>Chronic</td>
<td>347</td>
<td>162</td>
<td>185</td>
<td>185</td>
<td>100%</td>
<td>0</td>
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<tr>
<td>Recovery</td>
<td>88</td>
<td>63</td>
<td>25</td>
<td>25</td>
<td>100%</td>
<td>0</td>
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<tr>
<td>TOTAL</td>
<td>813</td>
<td>397</td>
<td>416</td>
<td>32</td>
<td>100%</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reproducibility</th>
<th>Within run</th>
<th>Between run</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen Type</td>
<td>Runs</td>
<td>Mean OD</td>
</tr>
<tr>
<td>Weak positive</td>
<td>10</td>
<td>0.45</td>
</tr>
<tr>
<td>Moderate positive</td>
<td>10</td>
<td>1.53</td>
</tr>
<tr>
<td>Strong positive</td>
<td>10</td>
<td>2.3</td>
</tr>
<tr>
<td>Positive control</td>
<td>10</td>
<td>2.4</td>
</tr>
</tbody>
</table>
Resources

Troubleshooting

✓ Values of the Positive or Negative controls, which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones.

✓ If after mixing of the Chromogen A and B solutions into the wells, the, the color of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

References

### Plate Layout

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
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