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IVD



96 tests

REF

1877-12

# AccuDiag™ HEV-Ab ELISA

## Hepatitis E

REF 1877-12

ELISA KIT FOR TOTAL ANTIBODIES TO HEPATITIS E VIRUS  
Two-Step Incubation, Double Antigen Sandwich Principle

Test	HEV -Ab ELISA
Method	<b>ELISA: Enzyme Linked Immunosorbent Assay</b>
Principle	<b>Sandwich ELISA: Double Antigen</b>
Detection Range	<b>Qualitative Positive; Negative control &amp; Cut off</b>
Sample	<b>50ul Serum</b>
Specificity	<b>99.60%</b>
Sensitivity	<b>99.80%</b>
Total Time	<b>~ 70 min</b>
Shelf Life	<b>12 Months from the manufacturing date</b>

*\* Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

## **INTENDED USE**

The purpose of the HEV Ab ELISA Test is for use as an aid in laboratory research diagnosis of zoonosis and human transmitted hepatitis E virus. The HEV Ab ELISA test is an enzyme-linked immunosorbent assay for the qualitative identification of total antibodies (IgG, IgM, etc.) to hepatitis E virus in animal serum/plasma. The HEV Ab ELISA Kit is for in-vitro diagnostic use only.

## **SUMMARY**

Hepatitis E is a recurring infection in developing countries. In less frequent, isolated cases, it has also been reported in developed countries. Identified in 1990, Hepatitis E (HEV) is a non-enveloped, single-stranded RNA virus where infection can lead to acute (or subclinical) liver disease, like that associated to hepatitis A infection. Fatality rates are generally low - averaging 0.5-3 percent for most patients, while pregnant women hold elevated averages around 15-25 percent. In 1995 a theory was introduced that HEV was transmitted to humans from animals (zoonosis). Not long after that, in 1997 and 2001, respectively, HEV was identified as transmitted from swine and from birds. After that time, HEV infection transmission has been associated with several animals, for example, wild monkeys, deer, cow, goats, rodents, dogs, and chickens. Found in both developing and developed countries, these HEV infections (including anti-HEV) consisted of viremia and feces excretion of HEV-affected animals, such as those listed above. A direct link to acute hepatitis E in humans from eating uncooked deer meat, was also reported. Also found in supermarkets in Japan, HEV genome sequences have been identified in pork livers. Serology has been enhanced in relation to the discovery of conformational epitopes in HEV. Diagnosis, epidemiology, zoonosis-related studies, and development of a vaccine have all been strengthened by the occurrence of long-lasting and protective HEV antibodies.

## **PRINCIPLE OF THE ASSAY**

The recombinant HEV antigens (HEV-Ag), corresponding to structural proteins ORF-2 of the native virus, are pre-coated on the polystyrene microwell strips of this HEV Ab ELISA test. The HEV Ab ELISA test uses serum or plasma specimen which are pipetted into these microwells. The pre-coated antigens will be bound to any HEV Ab present, and during the first incubation stage, the specific immunocomplex formed is captured on the solid phase. Next, it is important to wash the wells so that any unbound serum proteins can be removed. Added to the wells after this, is the second recombinant HEV antigen conjugated to Horseradish Peroxidase (HRP). This antigen, at the second incubation stage, will bind to the second variable domain of the HEV antibodies, if they have been captured by HEV-antigen at first incubation step. Next, the unbound HRP-conjugate is removed by washing the wells. Added to the wells are the chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide. This is done in the presence of the antigen-antibody-antigen (HRP) sandwich complex. A blue-colored product appears when the colorless chromogens are hydrolyzed by the bound HRP conjugate. The color intensity can be gauged proportionally to the amount of antibody captured in the wells, and to the sample, respectively. Colorless wells appear when samples are negative for HEV

# COMPONENTS



## 96 Tests

- **MICROWELL PLATE** 1plate  
Blank microwell strips fixed on a white strip holder.  
The plate is sealed in aluminium pouch with desiccant.  
8x12/12x8-well strips wells per plate. Each well contains recombinant HEV antigens.  
The microwell strips can be broken to be used separately.  
Place unused wells or strips in the plastic sealable storage bag together with the desiccant and return to 2~8°C.
- **NEGATIVE CONTROL** 1vial  
Blue-colored liquid filled in a vial with green screw cap  
0.5ml per vial.  
Protein-stabilized buffer tested non-reactive for HEV-Ab.  
Preservatives: 0.1% ProClin 300.  
Ready to use as supplied.  
Once open, stable for one month at 2-8°C.
- **POSITIVE CONTROL** 1vial  
Red-colored liquid filled in a vial with red screw cap  
0.5ml per vial.  
HEV-Ab diluted in protein-stabilized buffer  
Preservatives: 0.1% ProClin 300.  
Ready to use as supplied.  
Once open, stable for one month at 2-8°C.
- **SPECIMEN DILUENT** 1vial  
Blue-colored liquid filled in a white vial with blue screw cap.  
6 ml per vial.  
Protein-stabilized buffer, casein, and sucrose solution.  
Ready to use as supplied.  
Once open, stable for one month at 2-8°C.
- **HRP-CONJUGATE REAGENT** 1vial  
Red-colored liquid filled in a white vial with orange / red screw cap.  
12ml per vial.  
Horseradish peroxidase-conjugated recombinant HEV antigens.  
Ready to use as supplied.  
Once open, stable for one month at 2-8°C.
- **STOCK WASH BUFFER** 1bottle  
Colorless liquid filled in a clear bottle with white screw cap.  
50ml per bottle  
PH 7.4, 20 x PBS. (Containing Tween-20 as a detergent)  
**DILUTE BEFORE USE**-The concentration 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 when stored at 2-8°C.
- **CHROMOGEN SOLUTION A** 1vial  
Colorless liquid filled in a white vial with green screw cap.  
7 ml per vial.  
Urea peroxide solution. Ready to use as supplied.  
Once open, stable for one month at 2-8°C

- **CHROMOGEN SOLUTION B** 1vial  
 Colorless liquid filled in a brown vial with brown/black screw cap.  
 7 ml per vial.  
 TMB solution (Tetramethylbenzidine dissolved in citric acid).  
 Ready to use as supplied.  
 Once open, stable for one month at 2-8°C.
- **STOP SOLUTION** 1vial  
 Colorless liquid filled in a yellow vial with white/yellow screw cap.  
 7 ml per vial  
 Diluted sulfuric acid solution (0.5M H<sub>2</sub>SO<sub>4</sub>).  
 Ready to use as supplied.
- **PLASTIC SEALABLE BAG** 1unit  
 For enclosing the strips not in use.
- **CARDBOARD PLATE COVER** 2sheets  
 To cover the plates during incubation and prevent evaporation  
 or contamination of the wells.
- **PACKAGE INSERTS** 1copy

## **ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED BUT NOT PROVIDED**

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

## **SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE**

**1. 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 as 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 hemolyzed samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.

**2. Transportation and Storage:** Store samples at 2-8°C. Samples not required for assaying within 7 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.

## SPECIAL INSTRUCTION FOR WASHING

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. 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 of 350-400µl/well are sufficient to avoid false positive reactions and high background.
3. 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.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. 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.
5. In case of manual washing, we suggest to carry out 5 cycles, dispensing 350-400µl/well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before liquids are wasted in an appropriate way.
7. The concentrated Washing solution should be diluted **1 to 20** before use. For one plate, mix 50 ml of the concentrate with 950ml of water for a final volume of 1000ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.

## STORGE AND STABILITY

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 HEV-Ab ELISA kit, protect the reagents from contamination with microorganism or chemicals during storage.

## PRECAUTIONS AND SAFETY

This kit is intended **FOR IN VITRO USE ONLY** [IVD]

### 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.

1. 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.
2. 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.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature (18-30°C) before use.
4. Shake reagent gently before, and return to 2-8°C immediately after use.
5. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
6. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
7. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
8. 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.

9. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
10. 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.
11. Assure that the incubation temperature is 37°C inside the incubator.
12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from animal origin should be considered as potentially infectious.
15. 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.
16. 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.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for not less than 2hours at 121°C or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
18. The Stop solution (0.5M H<sub>2</sub>SO<sub>4</sub>) 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.
19. 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.
20. Materials Safety Data Sheet (MSDS) available upon request.
21. 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.

## ASSAY PROCEDURE

- Step1 Reagents preparation:** Allow the reagents and samples to reach room temperature (**18-30°C**) for at least 15-30minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the stock Wash Buffer **1 to 20** with distilled or deionized water. Use only clean vessels to dilute the buffer.
- Step2 Numbering Wells:** Set the strips needed in strip-holder and number sufficient number of wells including three Negative control (**e.g. B1, C1, D1**), two Positive control (**e.g. 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.
- Step3 Adding Diluent:** Add **50µl** Specimen Diluent into each well.
- Step4 Adding Sample:** Add **50µl** of Positive control, Negative control, and Specimen into their respective wells. **Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive as to avoid cross-contamination. Mix by tapping the plate gently.**
- Step5 Incubating Sample:** Cover the plate with the plate cover and incubate for **30minutes 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.
- Step6 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 the plate to remove any remainders.
- Step7 Adding HRP-Conjugate:** Add **100µl** HRP-Conjugate to each well except the Blank.
- Step8 Incubating:** Cover the plate with the plate cover and incubate the plate for **30 minutes at 37°C**



- Step9 Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash Buffer as in **Step6**.
- Step10 Coloring:** Dispense **50 µl** of Chromogen A and **50µl** Chromogen B solution into each well including the **Blank** and mix by tapping the plate gently. Incubate the plate at **37°C for 15minutes avoiding light**. The enzymatic reaction between the Chromogen A/B solutions produces blue color in Positive control and HEV-Ab positive sample wells.
- Step11 Stopping Reaction:** Using a multichannel pipette or manually add **50µl** Stop Solution into each well and mix gently by tapping the plate. Intensive yellow color develops in Positive control and HEV-Ab positive sample wells.
- Step12 Measuring the Absorbance:** 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:** read the absorbance within **10** minutes after stopping the reaction)

## INTERPRETATION OF RESULTS AND QUALITY CONTROL

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.

### 1. Calculation of Cut-off value (C.O.) = \*Nc + 0.12

\*Nc = the mean absorbance value for three negative controls.

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.

### 2. Quality control range:

The test results are valid if the Quality Control criteria are verified. 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.

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

**EXAMPLE:**

**1. Quality Control**

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

<b>Well No:</b>	<b>B1</b>	<b>C1</b>	<b>D1</b>
Negative controls A values after blanking:	0.020	0.012	0.016

<b>Well No:</b>	<b>E1</b>	<b>F1</b>
Positive control A values after blanking:	2.421	2.369

All control values are within the stated quality control range

**2. Calculation of Nc:** =  $\frac{(0.020+0.012+0.016)}{3} = 0.016$

**3. Calculation of Cut-off value:** Cut-off (C.O) = 0.016+0.12=0.136

**3. 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 antibodies to hepatitis E virus have been detected with this HEV-Ab ELISA kit.

**Positive Results (S/C.O. ≥1) :** samples giving an absorbance greater than or equal to the Cut-off value are considered initially reactive, which indicates that antibodies to hepatitis E virus have probably been detected using this HEV-Ab ELISA kit. Retesting in duplicates of any reactive sample is recommended. Repeatedly reactive samples could be considered positive for antibodies to HEV. Therefore there are serological indications for past or current infection with hepatitis E virus.

**Borderline (S/C.O. =0.9-1.1):** Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these samples in duplicates is recommended to confirm the results. Repeatedly positive samples could be considered positive for antibodies to HEV.

**TEST PERFORMANCE AND EXPECTED RESULTS**

**The prevalence studies of anti-HEV among animals with this HEV-Ab ELISA**

The prevalence of anti-HEV was highest in pigs, 83.34%; followed by this in cattle, 6.38%. Among 419 pig sera, the prevalence of anti-HEV and HEV viremia was 78.8% and 1.9% respectively, and five partial HEV genomic sequences were obtained and classified into Genotype-IV HEV.

The prevalence studies of anti-HEV among animals

Animal	No tested	No positive	Positive rate (%)
Pig	8628	7191	83.34
Cattle	392	25	6.38
Goat	370	5	1.35
Chicken	173	3	1.73

**LIMITATIONS**

1. Non-repeatable positive result may occur due to the general biological of the ELISA assays. This assay is designed to achieve very high performance characteristics of sensitivity and specificity and the “sandwich model” minimizes the unspecific reactions due to interference with unknown matters in sample. A negative result with an antibody detection test does not preclude the possibility of infection.
2. 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.



3. The prevalence of the marker will affect the assay's predictive values.
4. This is a qualitative assay and the results cannot be used to measure antibodies concentrations.

## **INDICATIONS OF INSTABILITY OR DETERIORATION OF THE REAGENTS**

1. 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.
2. If after mixing of the Chromogen A and B solutions into the wells, the color of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

## **VALIDITY**

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

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