



User's Manual

Human Anti-Hepatitis A Virus IgG (HAV-IgG) ELISA kit



DEIA007



96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Human HAV-IgG ELISA Kit is an enzyme linked-immunosorbent assay (ELISA) for qualitative detection of IgG-class antibodies to hepatitis A virus in human serum or plasma. It is intended for use in clinical laboratories for diagnosis and monitoring of patients related to infection with hepatitis A virus.

General Description

Hepatitis A is a self-limited disease and chronic stage or other complications are rare. Infections occur early in life in areas with poor sanitation and crowded living conditions. With improved sanitation and hygiene, infections are delayed and consequently the number of persons susceptible to the disease increases. Because the disease is transmitted through the fecal-oral route, in dense populated regions an outbreak can arise from single contaminated source. The cause of hepatitis A is hepatitis A virus (HAV)-non enveloped positive strand RNA virus with a linear single strand genome, encoding for only one known serotype. HAV has four major structural polypeptides and it localizes exclusively in the cytoplasm of human hepatocytes. The infection with HAV induces strong immunological response and elevated levels, first of IgM and then IgG are detectable within a few days after the onset of the symptoms. The serological detection of HAV antibodies is an important marker for confirming infection phase with HAV, classification of the virus and identifying the source of infection.

Principles of Testing

Human HAV-IgG ELISA Kit is based on solid phase, one-step incubation competitive principle ELISA method. If HAV IgG antibodies present in the sample, they compete with monoclonal HAV-IgG antibodies labeled with horseradish peroxidase (HRPConjugate) for a fixed amount of purified HAV antigens pre-coated in the wells. When no HAV-IgG presents in the sample, the HRP labeled HAV-IgG will be bound with the antigens inside the wells and the unbound HRP-Conjugate is removed during washing. Chromogen A and B solutions are added into the wells and during incubation, 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. No or low color developing suggests the presence of antibodies to HAV in the sample.

Reagents And Materials Provided

This kit contains reagents sufficient for testing of maximum of 91 specimens in a test run.

1. MICROWELL PLATE:

Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. Each well contains purified HAV antigens. The microwell strips can be broken to be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with the desiccant and return to 2-8°C. Once open, stable for one month at 2-8°C.

2. NEGATIVE CONTROL:

Yellowish liquid filled in a vial with green screw cap. Protein-stabilized buffer tested non reactive for HAV IgG.

Ready to use as supplied. Once open, stable for one month at 2-8°C.

3. POSITIVE CONTROL:

Red-colored liquid filled in a vial with red screw cap. HAV IgG antibodies diluted in protein-stabilized buffer. Ready to use as supplied. Once open, stable for one month at 2-8°C.

4. HRP-CONJUGATE:

Red-colored liquid in a white vial with red screw cap. Horseradish peroxidase-conjugated HAV IgG antibodies. Ready to use as supplied. Once open, stable for one month at 2-8°C.

5. WASH BUFFER:

Colorless liquid filled in a white bottle with white screw cap. PH 7.4, 20x PBS. 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 when stored at 2-8°C.

6. 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.

7. CHROMOGEN SOLUTION B:

Colorless liquid filled in a black vial with black screw cap. TMB (Tetramethyl benzidine) solution. Ready to use as supplied. Once open, stable for one month at 2-8°C.

8. STOP SOLUTION:

Colorless liquid in a white vial with white screw cap. Diluted sulfuric acid solution (0.5M H₂SO₄). Ready to use as supplied. Once open, stable for one month at 2-8°C.

9. PLASTIC SEALABLE BAG: For enclosing the strips not in use, 1 unit.

10. PACKAGE INSERT: 1 copy.

11. CARDBOARD PLATE COVER: 2 sheets.

To cover the plates during incubation and prevent evaporation or contamination of the wells.

Materials Required But Not Supplied

1. Freshly distilled or deionized water
2. Disposable gloves and timer
3. Appropriate waste containers for potentially contaminated materials
4. Dispensing system and/or pipette
5. Disposable pipette tips
6. Absorbent tissue or clean towel
7. Dry incubator or water bath, 37±0.5°C
8. Plate reader, single wavelength 450nm or dual wavelength 450/630nm
9. Microwell aspiration/wash system

Storage

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 the Human HAV-IgG ELISA Kit, during storage, protect the reagents from contamination with microorganism or chemicals.

Specimen Collection And Preparation

- 1. Specimen Collection: No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum or plasma specimens can be used with 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 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 specimen should be removed by centrifugation at 3000 RPM (round per minutes) 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 hemolytic specimens should not be used as they can 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. CD Human HAV-IgG ELISA Kit is intended ONLY for testing of individual serum or plasma samples. Do not use the assay for testing of 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 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 transportation of clinical samples and ethological agents.**

Reagent Preparation

Allow the reagents to reach room temperature (18-30°C). Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize 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 only clean vessels to dilute the buffer. All other reagents are **READY TO USE AS SUPPLIED**.

Assay Procedure

INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential in order 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 specimen or HRP-conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. 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 washing 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 they are wasted in an appropriate way.
7. The concentrated Wash buffer should be diluted 1 to 20 before use. If less than a whole plate is used, prepare the proportional volume of solution.

Procedure

1. Preparation: Mark three wells as Negative control (e.g. B1, C1, D1), two wells as Positive control (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 Sample: Add 50 µL of Positive control, Negative control, and Specimen into their respective wells except the Blank. Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination.

3. Adding HRP-Conjugate: Add 50 µL of HRP-Conjugate into each well except the Blank. Mix by tapping the plate gently.

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

5. 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.

6. Coloring: Add 50 µL of Chromogen A and 50 µL of Chromogen B solutions 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 color in Negative control and HAV-IgG negative sample wells.

7. Stopping Reaction: Using a multichannel pipette or manually, add 50 µL of Stop Solution into each well and mix gently. Intensive yellow color develops in Negative control and HAV-IgG negative sample wells.

8. 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**).

Quality Control

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.

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

Calculation

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 Cutoff 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.) = $N_c \times 0.5$

(N_c = the mean absorbance value for three negative controls).

Example:

1. Quality Control

Blank well A value: $A_1 = 0.025$ 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:	1.725	1.727	1.729

Well No.:	E1	F1
Positive control A values after blanking:	0.010	0.008

All control values are within the stated quality control range

2. Calculation of N_c : $= \frac{(1.725+1.727+1.729)}{3} = 1.727$

3. Calculation of the Cut-off: $(C.O.) = 1.727 \times 0.5 = 0.863$

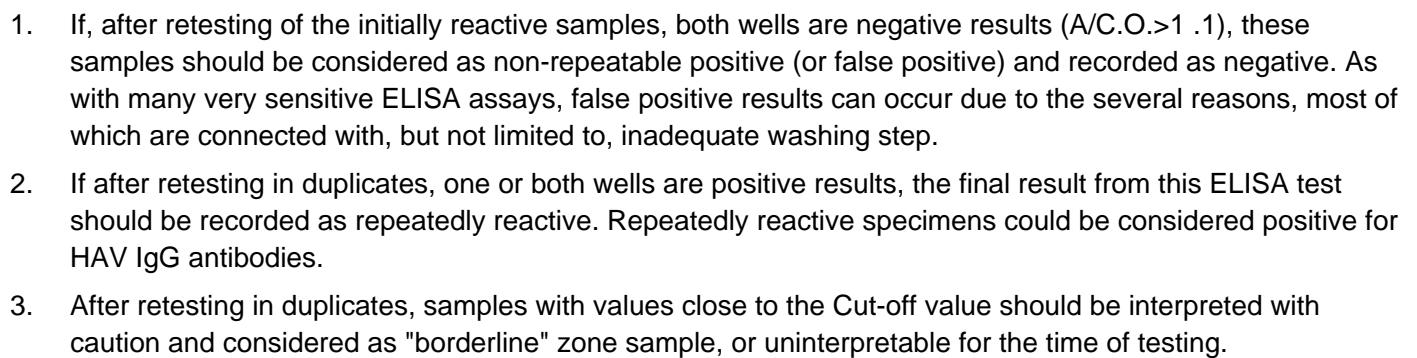
Interpretation Of Results

Negative Results ($A/C.O. > 1$): Specimens giving A value greater than the Cut-off value are negative for this assay, which indicates that no HAV IgG antibodies have been detected with the HAV-IgG ELISA, therefore there are no serological indications for past or current infection with hepatitis A virus.

Positive Results ($A/C.O. \leq 1$): Specimens giving A value less than or equal to the Cut-off value are considered initially reactive, which indicates that HAV IgG antibodies have probably been detected with the HAV-IgG ELISA. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for HAV IgG antibodies and therefore there are indications for past or current infection with hepatitis A virus.

Borderline ($A/C.O. = 0.9-1.1$): Specimens with A 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.

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.



Human Anti-Hepatitis A Virus IgG ELISA Kit

TO BE USED ONLY FROM QUALIFIED PROFESSIONALS

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 121°C 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. Materials Safety Data Sheet (MSDS) available upon request.
19. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.
20. The Stop solution 0.5M H₂SO₄ is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.
21. ProClinTM 300 0.1% used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

INDICATIONS OF INSTABILITY DETERIORATION OF THE REAGENT: Values of the Positive or 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 case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results and proven deterioration or instability of the reagents, immediately substitute the reagents with new one or contact CD technical support for further assistance.

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. Therefore, negative results obtained with the HAV-IgG ELISA are only indication that the sample does not contain detectable level of HAV IgG antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with HAV.
3. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as nonrepeatable (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 antibodies concentrations.

