Version 22-05/24

User's Manual

Human Anti-Hepatitis A Virus IgG ELISA Kit (Quantitative)

REF **DEIASL276**

Σ **96T**

RUO

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

The Human Anti-Hepatitis A Virus IgG ELISA Kit detects and quantifies anti-hepatitis A virus IgG in human serum or plasma of exposed or immunized animals. This immunoassay is suitable for:

(1) Determining immune status relative to non-immune controls;

(2) Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization and timing;

(3) Qualifying and/or standardizing vaccine batches and protocols.

This kit is for research use only (RUO), not for diagnosis or therapeutic purposes.

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 Hepatitis virus (HAV) is a Picornavirus; it is non-enveloped and contains a single-stranded RNA packaged in a protein shell. HAV has four major, structural polypeptides (VP1-4; 60 copies of VP1, 30-33 kD; VP2, 24-30 kD; VP3 (21-28 kD) 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. IgG is an indicator of past infection and immunity to HAV. HAV is detected by the presence of HAV antigens or antibodies using ELISA. HAV occurs endemically in all parts of the world.

Principles of Testing

The Human Anti- Hepatitis A Virus IgG ELISA kit is based on the binding of human anti- Hepatitis A IgG in samples to Hepatitis A antigen immobilized on the microwells, and anti- Hepatitis A IgG antibody is detected by anti-Human IgG specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti- Hepatitis A IgG present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The activity of human IgG antibody in samples is calculated relative to anti- Hepatitis A calibrators.

Reagents And Materials Provided

1. Hepatitis A Virus Coated Strip Plate: 8-well strips (12)
2. Anti-Hepatitis A Positive Control: 0.65 ml
3. Anti-Hepatitis A Calibrator (1 U/ml): 0.65 ml
4. Anti-Hepatitis A Calibrator (2.5 U/ml): 0.65 ml
5. Anti-Hepatitis A Calibrator (5 U/ml): 0.65 ml

6. Anti-Hepatitis A Calibrator (10 U/ml): 0.65 ml
7. Anti-Human IgG HRP Conjugate (100X): 0.15 ml
8. Sample Diluent (20x): 10 ml
9. Low NSB Sample Diluent: 30 ml
10. Wash Solution Concentrate (100X): 10 ml
11. TMB Substrate: 12 ml
12. Stop Solution: 12 ml
13. Product Manual: 1 ea

Materials Required But Not Supplied

1. Pipettors and pipettes that deliver 100ul and 1-10ml. A multichannel pipettor is recommended
2. Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Rabbit IgG HRP Concentrate.
3. Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
4. Distilled or deionized water to dilute reagent concentrates.
5. Microwell plate reader at 450 nm wavelength.

Storage

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

Specimen Collection And Preparation

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Antibody Stability & Dilution

Initial dilution of serum into Working Sample Diluent (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into Low NSB Sample Diluent (LNSD), which provides the lowest assay background, should be at least 5 times the initial dilution and performed the same week as the assay.

Example: Initial (1/5): 10ul serum + 40ul WSD [or 0.1ml + 0.4ml]

Further (1/50): 10ul initial (1/5) + 90ul LNSD (1/50)

Plate Preparation

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

1. Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample control to be assayed.

2. Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
3. Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Reagent Preparation

Wash Solution Concentrate (100x)

Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as Working Wash Solution and store at 4o C for long term and RT for short term.

Sample Diluent Concentrate (20x)

Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as Working Sample Diluent and store at 2-8 °C until the kit lot expires or is used up.

Anti-Human IgG- HRP Conjugate Concentrate (100x)

Peroxidase conjugated anti-Human IgG in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of Working Sample Diluent is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

Assay Procedure

Review Calculation of Results and Limits of the Assay before proceeding:

1. Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the 1 U/ml Calibrator. This is usually 1/100 or greater dilution for human serum with normal levels of IgG and IgM.
2. Run a Sample Diluent Blank. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.
3. Run the Anti-Hepatitis A Positive Control; the value range is on the label.
4. Run a set of Calibrators, which validate that the assay was performed to specifications: 10 U/ml should give a high signal (>1.5 OD); 1 U/ml should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results, p. 5).

Assay Steps

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

(1) Add 100ul of calibrators, samples and controls each to predetermined wells.

(2) Tap the plate gently to mix reagents and incubate for 60 minutes.

(3) Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2 nd Incubation [100ul – 30 min; 5 washes]

(1) Add 100ul of diluted Anti-Human IgG HRP to each well.

(2) Incubate for 30 minutes.

(3) Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

(1) Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.

(2) Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

(1) Add 100ul of Stop Solution to each well.

(2) Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

(1) Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.

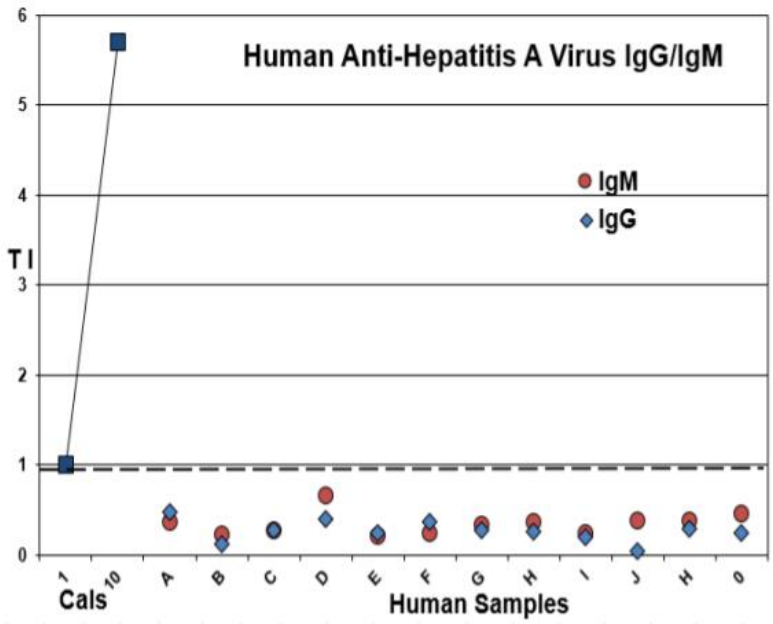
(2) Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

Interpretation Of Results

Example:

Human Serum IgG & IgM

A panel of sera/plasma from humans of unknown history was tested for anti- Hepatitis A Virus IgG & IgM (1:100 dilution in Low NSB Sample Diluent). Threshold Index was calculated using the 1 U/ml Calibrator.



Results

Anti-Hepatitis A Virus IgG & IgM:

Each of the twelve (12) human sera/plasma samples were negative (clearly below 1.0 TI) for both IgG & IgM antibodies.

Notes:

1. Positives may be due to prior encounter with the virus, from exposure to an antigen with common epitopes, or from immunization.
2. When the Positive Index is above 5.0, using a dilution curve to calculate titer is a more accurate quantitation method (see Method C).
3. The sensitivity of the assay may be adjusted by changing the sample dilutions: a) increase dilution (e.g., 1:200) to lower the signals of borderline positives to negative; b) decrease dilution (e.g., 1:50) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative threshold should be considered using known negatives to develop a Positive Index (see below) or use an Internal Control.

B. Positive Index

Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a Positive Index. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Nonimmune samples = Positive Index.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 are Negative for antibody.

A sample value would be Positive if significantly above the value of the pre-immune serum sample or a suitably determined nonimmune panel or pool of samples, tested at the same sample dilution.

This calculation also quantifies the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

Method C. Titers from Sample Dilution Curves

The titer of elevated antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
4. The Calibrator values can be used to normalize inter-assay values.

Calculations

1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index = IgG Antibody Activity Units

Sensitivity

Human Anti-Hepatitis A Virus IgG ELISA Kit

Specificity

Hepatitis A viral particles purified from infection of FRhK4 cells is used to coat the microwells; thus the assay is specific for antibodies directed to the Hepatitis A virus. The Anti-Human IgGHRP conjugate reacts specifically with human IgG class antibodies; IgA, IgM and IgE antibody would not be measured above background signals.

Precautions

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.