Datasheet for ABIN1305202

HAV IgG ELISA Kit

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Overview

Quantity:	96 tests
Target:	HAV IgG
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	This ELISA is a direct and quantitative enzyme immunoassay for the detection of human antibody (specifically for IgG subtype) against hepatitis A virus in human specimen.
Sample Type:	Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	1. HAV Antigen Coated Microplate (30338) One microplate with 12 x eight strips (96 wells total) coated with inactive HAV antigen. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2-8 °C and is stable until the expiration date on the kit box. 2. HAV Tracer Antibody (30339) One vial containing 0.6 mL concentrated horseradish peroxidase (HRP) conjugated anti-human IgG tracer antibody in a stabilized protein matrix. This reagent must be diluted with Tracer Antibody Diluent before use. This reagent should be stored at 2-8 °C and is stable until the expiration date on the kit box. 3. HAV Tracer Antibody Diluent (30340)

	One vial containing 12 mL ready to use buffer. It should be only used for antibody dilution according to the assay procedures. This reagent should be stored at 2-8 °C and is stable until the expiration date on the kit box. 4. ELISA HRP Substrate (10020) One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2-8 °C and is stable until the expiration date on the kit box. 5. ELISA Stop Solution (10030) One bottle contains 12 mL of 0.5 M sulfuric acid. This reagent should be stored at 2-8 °C or room temperature and is stable until the expiration date on the kit box. 6. HAV Standards (30349 ? 30353) Five vials each contain 1 ml of HAV antibody in a liquid bovine serum albumin based matrix with a non-azide preservative. Refer to vial for exact concentration for each calibrator. After the first use, the calibrators should be stored at -20 °C or below for long term storage. 7. HAV Controls (30354 ? 30355) Two vials each contain 1 ml of HAV antibody in a liquid bovine serum albumin based matrix with a non azide preservative. Refer to vials for exact concentration range for each control. After the first use, the calibrators should be stored at -20 °C or below for long term storage. 8. HAV Assay Buffer Concentrate, 20x (30348) One bottle contains 20 mL phosphate buffer with protein stabilizers and preservative. This reagent is 20 fold concentrate. It must be diluted with 380 DI-water or DT-water before use. This reagent should be stored at 2-8 °C and is stable until the expiration date on the kit box. 9. ELISA Wash Concentrate (10010) One bottle contains 20 mL of 30 fold concentrate. Before use the contents must be diluted with 580 mL of distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide preservative. The diluted wash buffer should be stored at room temperature and is stable until the expiration date on the kit box.
Material not included:	1. Precision single channel pipettes capable of delivering 10 µL to 100 µL, and 1000 µL. 2. Repeating dispenser suitable for delivering 100 µL. 3. Disposable pipette tips suitable for above volume dispensing. 4. Disposable 12 x 75 mm glass or plastic tubes. 5. Disposable plastic 1000 mL bottle with caps. 6. Aluminum foil. 7. Plastic microtiter well cover or polyethylene film. 8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.

Product Details

	9. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

Target Details

Target:	HAV IgG
Abstract:	HAV IgG Products
Target Type:	Antibody, Antibody
Background:	Hepatitis A, (formerly known as infectious hepatitis), is an acute infectious disease of the liver caused by Hepatitis A virus, which is most commonly transmitted by the fecal-oral route via contaminated food or drinking water. Every year, approximately 10 million people worldwide are infected with the virus. The time between infection and the appearance of the symptoms, (the incubation period), is between two and six weeks and the average incubation period is 28 days. Hepatitis A infection causes no clinical signs and symptoms in over 90 % of these children and since the infection confers lifelong immunity, the disease is of no special significance to the indigenous population. Hepatitis A does not have a chronic stage and does not cause permanent liver damage. Following infection, the immune system makes antibodies against the hepatitis A virus that confer immunity against future infection. The disease can be prevented by vaccination and hepatitis A vaccine has been proved effective in controlling outbreaks worldwide. The Hepatitis virus (HAV) is a picornavirus, it is non-enveloped and contains a single-stranded 27 nm RNA packaged in a protein shell. There is only one type of the virus. Although the virus is excreted in the feces towards the end of the incubation period, specific diagnosis is made by the detection of Hepatitis A virus specific IgM antibodies in the blood. IgM antibody is present in the blood following an acute hepatitis A infection. It is detectable from one to two weeks after the initial infection and persists for years. The presence of IgG antibody in the blood means that the acute stage of the illness is past and the person is immune to further infection. IgG antibody to HAV is also found in the blood following vaccination and tests for immunity to the virus are based on the detection of this antibody. During the acute stage of the infection the liver enzyme alanine transferase (ALT) is present in the blood at levels much higher than is normal. The enzyme comes from the liver cells that have been damaged by the virus.

Application Details

Assay Time:	4 h
Plate:	Pre-coated

Application Details

Protocol:	The quantitative HAV antibody (IgG) ELISA is a solid phase direct immunoassay to detect IgG antibody against HAV. Microwells are coated with both HAV multiple epitope synthetic peptide and recombinant antigen. Assay standards, controls and diluted unknown serum or plasma specimen are added to the microwells. After an incubation period, the unbound antibody are washed away, and a horseradish Peroxidase (HRP) conjugated-rabbit anti-human IgG is added to each well. The immunocomplex of well bound HAV antigen-human anti-HAV IgG antibody-HRP conjugated anti-human IgG will be formed. The unbound enzyme conjugates will be washed away and then the chromogen substrate solution containing urea peroxide is added to all the wells. A blue color is developed with the color intense in proportion to the amount of anti-HAV IgG antibody in the specimens. The enzyme-substrate reaction is stopped by the addition of sulfuric acid. The absorbance of assay standards, controls and unknown specimens are determined by an EIA plate reader with wavelength set at 450 nm.
Reagent Preparation:	(1) Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged. (2) ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details. (3) HAV Assay Buffer Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.
Sample Collection:	Either serum or plasma can be used in this test. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected by venipuncture and must be allowed to clot for a minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 ? 1500xg for 10 minutes). The serum should be separated from the clot within two hours of blood collection and transferred to a clean test tube. Serum samples should be stored at 2 - 8 C if the assay is to be performed within 24 hours. Otherwise, patient samples should be stored at -20 °C or below until measurement. Avoid any repeated freezing and thawing of specimen. Grossly hemolytic, lipidic or turbid samples may interfere test results and should not be used.
Assay Procedure:	(1) Place a sufficient number of HAV antigen coated microwell strips in a holder to run assay controls and unknown samples in duplicate. (2) Test Configuration (3) Dilute each unknown specimen in 1:4,000 before the specimen being assayed. It is suggested to do a two-step dilution for each specimen. For example, one can mix 1000 ?l of assay buffer with 15.8 ?l of unknown specimen in a clean tube (D1) and further mix 1000 ?l of assay buffer with 15.8 ?l of the prediluted specimen from D1 (D2). The diluted sample (D2) is ready to be measured in the following assay procedures.

	(4) Add 100 ?l of assay standards, controls and the diluted unknown specimens into respective wells. (5) Incubate the plate at room temperature (22 ? 25 C) and shaking (~400 rpm) for 40 min. (6) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used. (7) Prepare Tracer Antibody Working Solution by 1:21 fold dilution of the tracer antibody with the Tracer Antibody Diluent. For each strip, it is required to mix 1 mLof Tracer Antibody Diluent with 50 µL of Tracer Antibody in a clean test tube. (8) Add 100 ?l of above diluted HAV Tracer Antibody Working Solution to each well. (9) Incubate the plate at room temperature (22 ? 25 C) and shaking (~400 rpm) for 40 min. (10) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used. (11) Add 100 µL of ELISA HRP Substrate into each of the wells. (12) Cover the plate with an aluminum foil to avoid exposure to light. (13) Incubate plate at room temperature (22 ? 25 C) and static for 20 minutes (14) Remove the aluminum foil and plate sealer. Add 100 µL of ELISA Stop Solution into each of the wells. Mix gently. (15) Read the absorbance at 450 nm within 10 minutes in a microplate reader NOTE: to reduce the background, one can set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 595 nm or 620 nm or 630 nm.
Calculation of Results:	1. Calculate the average absorbance for each pair of duplicate test results. 2. Subtract the average absorbance of the calibrator 1 (0 IU/mL) from the average absorbance of all other readings to obtain corrected absorbance. 3. The calibrator curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results. The HAV antibody concentrations for the controls and samples are read directly from the calibration curve using their respective corrected absorbance. If log-log graphic paper or computer assisted data reduction program utilizing logarithmic transformation are used, sample having corrected absorbance between the 3.1 U/mL calibrator and the next highest calibrator should be calculated by the formula: Corrected absorbance (unknown) Value of unknown = x Value of the 2nd STD Corrected Absorbance (2nd STD)
Restrictions:	For Research Use only

Handling

Precaution of Use:	The reagents must be used in research laboratory and are for research use only. Reagents of bovine serum were derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potential infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. Upon contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.
Storage:	4 °C