

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	#1200	Human Albumin (Urinary)
#1750	Human IgG (total)	#1760	Human IgM
#1800	Human IgE	#1810	Human Ferritin
#1210	Human Transferrin (Tf)		
#0020	Beta-2 microglobulin		
#1600	Human Growth Hormone (GH)		
#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	#1830	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	#1510	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		
#0030	Human Insulin	#0040	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#0410	HCG-free beta
#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	#1110	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	#1700	Human T3 (total)
#1850	Human Cortisol	#1860	Human Progesterone
#1865	Human Pregnenolone	#1875	Human Aldosterone
#1880	Human Testosterone	#1885	Human free Testosterone
#1910	Human Androstenedione	#1920	Human Estradiol
#1925	Human Estrone	#1940	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		
#3000	Human Rheumatoid Factors IgM (RF)		
#3100	Human anti-dsDNA		
#3200	Anti-Nuclear Antibodies (ANA)		

Human Anti-Hepatitis A Virus (HAV-IgG)

ELISA KIT # 4300-AHG, 96 Tests

For Qualitative Determination of HAV IgG In Human
Serum or Plasma

For In Vitro Research Use Only



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Human Anti-HAV IgG ELISA kit # 4300-AHG Kit Contents (96 tests):

Components	#
HAV-Ag coated microwell strips (96 wells, 12 x 8 wells), #4301	1 Plate
HAV-IgG Positive Control , #4302, 0.5 ml	1 vial
HAV-IgG Negative Control , #4303, 1 ml	1 vial
Anti-Human IgG HRP conjugate (ready-to-use) , #4300-HRP, 6.5 ml	1 bottle
TMB substrate A , # 4300-TM-A, 7 ml	1 bottle
TMB substrate B , # 4300-TM-B, 7 ml	1 bottle
Stop Solution , #4300-ST, 6 ml	1 bottle
Wash buffer (40X) , #4300-WB, 20 ml	1 bottle
Complete Instruction Manual , M-4300-AHG	1

Intended Use

The kit is an enzyme linked-immunosorbent assay (ELISA) for qualitative determination of IgG-class antibodies to human hepatitis A virus (HAV-IgG) in 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 Information

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. IgG is an indicator of past infection and immunity to HAV. The serological detection of HAV IgG is an important marker for confirming infection phase with HAV, classification of the virus and identifying the source of infection.

Data Analysis

blank well: $A \leq 0.08$

Negative control: $A \leq 0.1$ (If one well $A > 0.1$, it should be abandoned; If two wells $A > 0.1$, it should be retested)

Positive control: $A \geq 0.8$

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.

Calculation of the Cutoff Value

Cutoff Value = $NCx + 2.1$

NCx: Mean Absorbance of Negative Control (when $NCx \leq 0.05$, regard as 0.05)

Calculation of Results

Sample with absorbance values \leq Cutoff Value is NON-REACTIVE and are considered NEGATIVE for HAV-IgG .

Sample with absorbance values $>$ Cutoff Value are considered POSITIVE for HAV-IgG .

ASSAY PROCEDURE

1. Put the kit at room temperature for **30 minutes** before use.
2. Wash plate 2 times with **350 ul** wash buffer before adding sample and control wells. Label the sample wells, 2 Negative Controls, 1 Positive Control and 1 blank well.
3. Add **50 µL** sample, negative controls and positive control to well (keep one as blank well), gently tap the plate to ensure thorough mixing. Cover the plate and incubate at **37°C for 30 min**.
4. Remove the cover, and wash plate **5 times** with **350 ul** Wash buffer and let the wash buffer stay in the wells for 1 minute each time.
5. Add **50 µL** Anti- human IgG HRP conjugate into each well, except blank well; gently tap the plate to ensure thorough mixing.
6. Cover the plate with and incubate at **37°C for 30 min**.
7. Remove the cover, and wash plate **5 times** with **350 ul** Wash buffer and let the wash buffer stay in the wells for 1 minute each time.
8. Add **50 µl** of TMB **substrate A** and **50 µl** of TMB **substrate B** into each well; except blank well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in dark for 10 min. And the shades of blue can be seen in the Positive Controls. Negative Controls wells show no obvious color.
9. Add **50 µl** of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution. (Use the blank well to set zero)

PRINCIPLE OF THE TEST

Human HAV-IgG ELISA kit was based on Indirect ELISA method. HAV-Ag was pre-coated onto 96-well plates. The test samples were added to the wells, unbound conjugates were washed away with wash buffer. Then added HRP conjugated anti-human-IgG, if there were any HAV-IgG in the samples, it would form a HAV-Ag –HAV-IgG- HRP Anti- human IgG complex. TMB substrates were used to visualize HRP enzymatic reaction. It was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450 nm with a selected reference wavelength within 650 nm.

MATERIALS AND EQUIPMENT REQUIRED

Microplate reader (wavelength: 450nm), 37°C incubator, Automated plate washer, Precision single and multi-channel pipette and disposable tips, Clean tubes and Eppendorf tubes, Deionized or distilled water.

SPECIMEN COLLECTION AND STORAGE

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

Serum: Coagulate the serum at room temperature (about 1 hours). Centrifuge at approximately 1000 x g for 15 min. Analyze the serum immediately or aliquot and store at -20°C.

Plasma: Collect plasma with heparin or EDTA as anticoagulant. Centrifuge for 15 min at 2-8°C at 1500xg within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000xg. Analyze immediately or aliquot and store at -20°C.

Notes:

1. Samples should be used within 5 days when stored at 2-8°C, otherwise samples must be divided and stored at -20°C (≤1month) or -80°C (≤6months) to avoid the loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.
2. Please take the samples to room temperature (18-25°C) without extra heating before performing the assay.
3. Hemolyzed samples are not suitable for use in this assay.

REAGENTS PREPARATION FOR THE ASSAY:

Wash Buffer (40X): Dilute the wash buffer 1:40 with distilled water (dissolve content of 1 bottle (20 ml) into 800 ml deionized or distilled water). Some buffer components may crystallize in wash concentrate. These redissolve at room temperature. Store diluted wash buffer at 2-8°C.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. Do not freeze and thaw.

Special Instructions 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 with dispensing of 350-400µl/well, are sufficient to avoid false positive reactions and high background (all wells turn yellow).
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's 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 perform at least 5cycles, dispensing 350-400µl/well and aspirating the liquid for 5times. 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(final concentration of 2.5%) for 24 hours, before liquids are disposed in an appropriate way.

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. Shake reagent gently before, and return to 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 microwell reading.
6. 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.
7. 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.
8. The use of automatic pipettes is recommended. Assure that the incubation temperature is 37°C inside the incubator.
9. When adding samples, avoid touching the well's bottom with the pipette tip.