ELISA kits available from ADI (see details at the web site)

#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 Pregnolone **#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)

Instruction Manual No. M-4300-AHG

Human Anti-Hepatitis A Virus (HAV-IgG)

ELISA KIT # 4300-AHG, 96 Tests

For Qualitative Determination of HAV IgG In Human Serum

For In Vitro Research Use Only

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

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, 1 ml	1 vial
HAV-IgG Negative Control, #4303, 1 ml	1 vial
Sample dilution buffer, #4304, **12 ml**	1 bottle
Anti-Human IgG HRP solution (ready-to-use), #4300-HRP, **12 ml**	1 bottle
TMB substrate A, # 4300-TM-A**, 6 ml**	1 bottle
TMB substrate B, # 4300-TM-B**, 6 ml**	1 bottle
Stop Solution, #4300-ST, **6 ml**	1 bottle
Wash buffer (20X), #4300-WB, **50 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. 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 ≤0.08

Negative control: A ≤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 ≥ 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 +0.1
NCx: Mean Absorbance of Negative Control (when A≤ 0.05, regard as 0.05)

Calculation of Results

Sample with absorbance values ≤ 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. Label the sample wells, 3 Negative Controls, 2 Positive Controls and 1 blank well, directed add 100ul contrast serum into each well (do not add sample dilution).
3. Add **100 µL** sample dilution to each well and then add **10 ul** sample, gently tap the plate to ensure thorough mixing. Cover the plate and incubate at **37℃ 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 **100 µL** Anti- human IgG HRP solution into each well, except blank well; gently tap the plate to ensure thorough mixing.
6. Cover the plate with and incubate at **37℃ 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. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37℃ in dark within 15 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℃ 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℃ for long term. Avoid multiple freeze-thaw cycles.

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

Notes:

1. Samples should be used within 5 days when stored at 2-8℃, otherwise samples must be divided and stored at -20℃ (≤1month) or -80℃ (≤6months) to avoid the loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.
2. Please take the samples to room temperature (18-25℃) 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 (20X): Dilute the wash buffer 1:20 with distilled water (dissolve content of 1 bottle (50 ml) into 950 ml deionized or distilled water). Some buffer components may crystallize in wash concentrate. These redissolve at room temperature. Store diluted wash buffer at 2-8oC.

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℃) before use. Shake reagent gently before, and return to 2-8℃ 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℃ inside the incubator.

9. When adding samples, avoid touching the well's bottom with the pipette tip.