bi-x-act® of FINLAND

Anti-HAV IgG ELISA 96 and 480 Well Plate Test

CE IVD

Productcodes: 2HAVG96
2HAVG480

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1.	**Intended Use**
	Anti-HAV IgG ELISA Test is an enzyme immunoassay for in vitro qualitative detection of antibody to hepatitis A virus (Anti-HAV) in human serum or plasma (heparin, EDTA or citrate).
2.	**Summary and Test Explanation**
	The hepatitis A virus (HAV) is a single-stranded RNA-containing virus without an envelope and with a diameter of 27 nm that belongs to the family of Picornaviridae (1). Hepatitis A - the most common form of acute viral hepatitis - is an infection of fecal-oral transmission produced in humans after an average incubation period of 28 days (range, 15-50 days). The illness caused by HAV infection typically has an abrupt onset of symptoms that can include fever, malaise, anorexia, nausea, abdominal discomfort, dark urine, and jaundice (2). Total anti-HAV and especially IgM anti-HAV is positive at the onset of a hepatitis A infection. After natural infection, anti-HAV-IgG antibodies can usually be detected for a lifetime providing protection against the disease (3-4). The detection of anti-HAV is indicative of current immunity and helps in deciding whether active immunization should be supplied by vaccination or immunoglobulins should be administered for post-exposure prophylaxis in at-risk situations (5-6). **Anti-HAV IgG ELISA Test** is a fast test for the qualitative detection of antibodies to Hepatitis A virus in serum or plasma (heparin, citrate or EDTA) specimens. This is an enzyme linked immunosorbent assay (ELISA) which utilizes HAV Ag on microtiter wells and human peroxidase-conjugated Anti-HAV in a competition principle to detect Anti-HAV levels in serum or plasma. Specimens with absorbance values greater than the Cutoff Value are considered **NONREACTIVE** for Anti-HAV Specimens with absorbance values lower or equal than the Cutoff Value are considered **REACTIVE** for Anti-HAV. The test has to be repeated in duplicate for specimens with absorbance value within the retest range (Cutoff Value ± 10 %) and interpreted as above. If the absorbance of any of the specimens retested in duplicate is still within the retest range, it is suggested to test follow-up samples of the patient.

3.	**Test Description**
	Anti-HAV IgG ELISA Test is a solid-phase enzyme immunoassay (ELISA= enzyme-linked immunosorbent assay) based on a competitive principle. The solid phase of the microtiter plate is made of polystyrene wells coated with HAV Ag and the liquid phase of human peroxidase conjugated Anti-HAV. When a serum or plasma specimen containing Anti-HAV is added to the HAV Ag-coated wells together with the human peroxidase conjugated Anti-HAV and incubated, a competition will take place for the binding to the HAV Ag on the wells. (HAV Ag)- (Anti-HAV • peroxidase) complex and/or (HAV Ag)-(Anti-HAV) complex will form on the wells. After washing the microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. Due to the competitive principle a color develops inversely proportional to the amount of Anti-HAV bound to HAV Ag deriving from the specimen. The peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450 nm with a selected reference wavelength within 620 to 690 nm (7).

A Specimen containing Anti-HAV:
1. Plate well (HAV Ag) + specimen (Anti-HAV) + Anti-HAV·peroxidase
→ Plate-HAV Ag-Anti-HAV complex and/or Plate-HAV Ag-Anti-HAV·peroxidase complex
2. Washing to remove unbound material
3. Add TMB substrate solution → blue color to light pale blue color/even colorless
2. Add sulfuric acid to stop the color development → Read OD at 450nm with a selected reference wavelength within 620 to 690nm[*7]

B Specimen without Anti-HAV:
1. Plate well (HAV Ag) + specimen (without Anti-HAV) + Anti-HAV·peroxidase
→ Plate-HAV Ag-Anti-HAV·peroxidase complex
2. + TMB substrate solution → colorless to blue to light blue color
3. Add 2N sulfuric acid to stop the color development, read OD at 450nm with a selected reference wavelength within 620 to 690nm[*7]

4.	**Description of Materials Provided & Product Code System**

Storage Conditions: Item 1 - 6 on the following reagent table should be refrigerated at +2 to 8+ °C and the others stored at room temperature (+20 to +30 °C).

ITEMS	Components	Description	Qt. per 96 tests
(1)	HAV Ag Test Plate	Microtiter Plate Coated with HAV Antigen.	1 plate
(2)	Anti-HAV·Peroxidase Solution	Anti-HAV (mouse monoclonal)·peroxidase (horseradish) conjugate dissolved in buffer with protein stabilizers. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.	1bottle, 11 ml
(3)	Anti-HAV Positive Control	Human plasma positive for antibody to HAV in buffer with protein stabilizers. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.	1 bottle, 0.5 ml
(4)	HB Negative Control	Human plasma non-reactive for antibody to HAV with protein stabilizers. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.	1 bottle, 0.5 ml
(5)	TMB Substrate Solution A	0.6 mg/ml of 3,3',5,5'-tetramethylbenzidine (TMB) in an organic base.	1 bottle, 10 ml
(6)	TMB Substrate Solution B	Citric Acid Buffer containing 0.03% H_2O_2.	1 bottle, 10 ml
(7)	Conc. Washing Solution D (20X)	Concentrated phosphate buffer with Tween-20.	1 bottle 52 ml
(8)	2$\underline{N}$ H_2SO_4	2$\underline{N}$ H_2SO_4 (Sulfuric Acid)	1 bottle 12 ml

ACCESSORIES: (provided as needed)

ITEMS	Components
(9)	Adhesive Slips
(10)	Absorbent Pads
(11)	Black Cover

OTHER MATERIALS AND DEVICES REQUIRED, BUT NOT PROVIDED

ITEMS	Components
(1)	10μl, 100μl and 1.0 ml micropipettes and tips are needed
(2)	Incubator or waterbath with temperature control at +37 °C.
(3)	Plate washing equipment.
(4)	ELISA microwell reader: Dual wavelength 450nm with 620-690nm as reference wavelength[*7], bandwidth 10nm
(5)	Purified water: distilled or deionized water.
(6)	Fully automatic EIA micro-plate analyzer is optional. User should validate the automatic EIA micro-plate analyzer in combination with the kit.

4.1.	Storage Conditions and Stability of Kit and Components *			
	Kit/Components	**Storage condition**	**State**	**Stability**
	Anti-HAV IgG ELISA Test Kit	+2～+8 °C	Original	15 months
			Once open	1 month
	Anti-HAV Positive Control	+2～+8 °C	Original	15 months
			Once open	1 month
	HAV Negative Control	+2～+8 °C	Original	15 months
			Once open	1 month
	HAV Ag Plate	+2～+8 °C	Original	15 months
			Once open	2 months
	Anti-HAV·Peroxidase Conjugate Solution	+2～+8 °C	Original	15 months
			Once open	1 month
	Concentrated Washing Solution D (20x)	+2～+8 °C	Original	24 months
			Once open	1 month
	20x Diluted Washing Solution	Room temp.	Diluted	2 days
		+2～+8 °C	Diluted	1 week
	TMB Substrate Solution A	+2～+8 °C	Original	18 months
			Once open	1 month
	TMB Substrate solution B	+2～+8 °C	Original	18 months
			Once open	1 month
	2N Sulfuric Acid	Room temp.	Original	24 months
			Once open	1 month

5.	Instructions for Use
5.1.	**Warnings**
5.1.1.	This reagent kit is for professional use only.
5.1.2.	This reagent kit is for *in vitro* diagnostic use only.

5.1.3.	Bring all kit reagents and samples to room temperature (+20 to +30°C) and mix gently before use.
5.1.4.	Do not use reagent beyond its expiration date.
5.1.5.	Do not interchange reagents between different lots.
5.1.6.	Do not pipette in the mouth.

5.1.7.	Do not smoke or eat in areas where specimens or reagents are handled.
5.1.8.	The positive control, negative control, conjugate solution and specimens should be regarded as potential hazards to health. They shall be used and discarded according to the user's laboratory safety procedures. Such safety procedures probably shall include wearing protective gloves and avoiding aerosols generation.
5.1.9.	Potential infectious specimens and nonacid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with the laboratory's practice for potential bio-hazard control.
5.1.10.	**Prior to dispose the waste of used specimens and kit reagents as general waste, it should be treated in accordance with the local procedures for potential bio-hazardous waste or treated as follows:** Both liquid and solid waste should be autoclaved maintaining +121 °C for at least 30 minutes. Solid waste can also be incinerated. Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%. Acidic liquid wastes must be neutralized before treatment with sodium hypochlorite as mentioned above and should stand for 30 minutes to obtain effective disinfection.
5.1.11.	2N sulfuric acid is an irritant to skin, eyes, respiratory tract and mucous membranes. Avoid contact of the 2N sulfuric acid with skin and mucous membranes. In case of contact, clean with large lots of water immediately. In case of inhalation, supply fresh air and seek medical advice in case of complaints.
5.1.12.	TMB substrate solution A contains an organic solvent, which is flammable. TMB substrate solution A contains dimethyl sulfoxide, an irritant to skin and mucous membranes.

5.2.	**Specimen Collection and Preparation for Analysis**
5.2.1.	No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques.
5.2.2.	Either serum or plasma can be used with this diagnostic kit. Whole blood specimens should be separated as soon as possible in order to avoid hemolysis. Any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen should be removed prior to use.
5.2.3.	Specimens must be stored at +2 to +8 °C and avoided heat-inactivation to minimize deterioration. For long-term storage, they should be frozen below -20 °C. Storage in self-defrosting freezer is not recommended.
5.2.4.	Frozen specimens must be thoroughly thawed and mixed homogenously before test.
5.2.5.	Avoid multiple freeze-thaw procedures
5.2.6. **WARNINGS**	1. The specimen must not contain any compounds of AZIDE, which inhibits the peroxidase activity.

	2. Incompletely coagulated serum samples and microbial-contaminated specimens should not be used.

5.3.	**Reagents storage**
5.3.1.	The kit must be stored at +2 to +8°C. Do not freeze.
5.3.2.	Strips of the plate should be used within 2 months after open the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag and taped the opening tightly.
5.3.3.	Return the reagents to +2 to +8 °C immediately after use.
5.3.4	Washing Solution D (20x) Concentrate should be stored at room temperature to avoid crystallization. If the crystal has been precipitated before use, warm up the solution in a +37 °C water bath till the crystal is dissolved.

5.4.	**Plate washing procedure**
5.4.1.	Preparation of washing solution: Dilute Washing Solution D (20x) Concentrate with distilled or de-ionized water to 1:20 dilution. Do not use tap water.
5.4.2.	Plate washing: Any commercial automatic micro-plate washer or other liquid aspirating/ dispensing devices can be used for washing purpose. The user should test the devices to determine the proper volume of water and wash cycles to insure proper washing. **It is suggested to wash 6 cycles with at least 350µl washing buffer per well per wash and soaking at least for 10 seconds.**
5.4.3.	Blot dry by inverting the plate and tapping firmly onto absorbent paper. Too much residual wash buffer will cause false results.
WARNING	Improper washing will cause false results.

5.5.	**Test procedure**
	Assay process can be performed by an automatic EIA micro-plate immunoanalyzer, Please set up the program according to the following test procedure.
5.5.1.	Bring all reagents and specimens to room temperature (+20 to +30°C) before assay. Adjust water bath or incubator to +37±1°C.
5.5.2.	Prepare the needed number of wells, including 2 wells for blanks, 3 wells for Negative Control, 2 wells for Positive Control, and one well for each specimen. Reserve 2 wells for blanks (**Do not add any specimen or conjugate)**. Add 10µl of each control or specimen to the appropriate wells of HAV Ag coated plate, except the 2 blanks. **NOTE:** a. Use a new pipette tip for each sampling to avoid cross-contamination b. Each plate needs its own negative controls, positive controls and blank wells. c. Do not use cut-off values established for other plates of **Anti-HAV IgG ELISA Test**
5.5.3.	Add 100µl of Anti-HAV·Peroxidase solution to each of the above wells

	except the 2 blanks. **Note:** Do not touch the cuvette wall for preventing contamination.
5.5.4.	Gently tap the plate.
5.5.5.	Seal the plate with an adhesive slip.
5.5.6.	Incubate the reaction plate in +37 ± 1 °C water bath or incubator for **one hour**.
5.5.7.	At the end of the incubation period, remove and discard the adhesive slip and wash the plate in accordance with **5.4) Plate washing procedure.**
5.5.8.	Choice one of the following two methods for color development: **NOTE:** TMB Substrate Solution A should be colorless to light blue; otherwise, it should be discarded. The mixture of TMB Substrate Solution A and B should be used within 30 minutes after mixing. The mixture should be avoided from intense light. A. Mix equal volumes of TMB Substrate Solution A and B in a clean container immediately prior to use. Add 100 µl of the mixture solution to each well including the 2 blank wells. B. Add 50µl of TMB Substrate Solution A first, then add 50µl of TMB Substrate Solution B into each well including the 2 blanks. Mix well gently.
5.5.9.	Cover the plate with black cover and incubate at room temperature for 30 minutes.
5.5.10.	Stop the reaction by adding 100 µl of 2N H_2SO_4 to each well including the blank.
5.5.11.	Determine the absorbance of controls and test specimens within 15 minutes with a photometer at 450nm with a selected reference wavelength within 620 to 690nm[7]. Use the blank well to blank the photometer. **NOTE:** The color of the blank should be colorless to light yellowish; otherwise, the test result is invalid. In this case the test must be repeated.

5.6.	**Calculation of Test Results**

5.6.1.	Calculation of the NCx (Mean Absorbance of Negative Control).
	Example:
	Sample No. Absorbance
	        1 1.263
	    2 1.305
	      3 1.290
	NCx = (1.263 + 1.305 + 1.290)/3=1.286
	NCx must be ≥ 0.4, otherwise, the test is invalid.

5.6.2.	Calculation of PCx (Mean Absorbance of Positive Control)
	Example:
	Sample No. Absorbance
	      1 0.054
	      2 0.060
	PCx = (0.054 + 0.060) /2 = 0.057
	PCx must be ≤0.1, otherwise, the test is invalid.

5.6.3.	Calculation of the **N-P Value**

	N-P = NCx – PCx Example: N - P = 1.286 - 0.057 = 1.229 **N-P Value must be ≥ 0.3, otherwise, the test is invalid.**
5.6.4.	Calculation of the **Cutoff Value** **Cutoff Value = (NCx + PCx)/2** Example: Cutoff Value =(1.286 + 0.057)/2 = 0.672
5.6.5.	Calculation of the **Retest Range** **Retest Range = Cutoff Value ±10%** Example: Cutoff Value = 0.672 Retest Range = (0.672 - 0.067) to (0.672 + 0.067) = 0.605 to 0.739
5.7.	**Validity of Test Runs**
5.7.1.	**NCx must be ≥0.4, otherwise, the test is invalid.**
5.7.2.	**PCx must be ≤0.1, otherwise, the test is invalid.**
5.7.3.	**N-P Value must be ≥0.3, otherwise, the test is invalid.**

5.8.	**Interpretation of Results**
5.8.1.	Specimens with O.D. values **GREATER** than the **Cutoff Value** are considered **non-reactive** for Anti-HAV.
5.8.2.	Specimens with O.D. values **LOWER** than or **EQUAL** to the **Cutoff Value** are considered **reactive** for Anti-HAV.
5.8.3.	If the data is within the **Retest Range**, the test must be repeated in duplicate and interpreted as above. If the retested absorbance still within the retest range, it is suggested to test follow-up-samples.
5.9.	**Troubleshooting**
	If the result cannot be reproduced, a preliminary troubleshooting should be performed by checking the possibilities listed below:
5.9.1.	Improper washing procedure.
5.9.2.	Contamination with positive specimens.
5.9.3.	Wrong volume of sample, conjugate or substrates.
5.9.4.	Contamination of well rim with conjugate.
5.9.5.	Improper specimen such as hemolyzed serum or plasma, specimen containing precipitate and specimen not being mixed well before use.
5.9.6.	Wrong incubation time or temperature.
5.9.7.	Obstructed or partial obstructed washer aspirate/dispense head and needles.
5.9.8.	Insufficient aspiration.

5.10.	**Limitations and Interferences**
5.10.1.	**This reagent kit is to be used for un-pooled human serum or plasma samples only.**
5.10.2.	**Non-repeatable reactive results may be obtained with any** enzyme immunoassay **kit,** largely due to technical error either on the part of the operator or malfunction of apparatus used.

5.10.3.	The reagent kit has not been validated for use with cadaveric samples.
5.10.4.	**Potential interfering substances: By addition tests the following results were obtained:** 1. The anticoagulants heparin, citrate and EDTA had no effect on the test result. 2. Hemoglobin up to 8.0 g/l had no effect on the test result. 3. Bilirubin up to 0.3 g/l: had no effect on the test result. 4. Triglyceride up to 5.0 g/l had no effect on the test result. 5. A rheumatoid factor high positive specimen exhibited a false positive result. 6. Pregnancy did not affect the test result.

5.11.	**Performance Characteristics**
5.11.1. **Diagnostic Sensitivity and Diagnostic Specificity**	1. Specimens from hospitalized patients:

1. Specimens from hospitalized patients:

		Anti-HAV IgG ELISA Test		
		negative	positive	Total
Comparison assay	Negative	984	5	989
	Positive	1	551	552
	total	985	556	1541

Diagnostic sensitivity = 100% x 551/552 = 99.8%
Diagnostic specificity = 100% x 984/989 = 99.5%

2. Patients with HAV infection

		Anti-HAV IgG ELISA Test		
		negative	positive	Total
Comparison assay	Negative	0	0	0
	Positive	0	19	19
	total	0	19	19

Diagnostic specificy=100%
Diagnostic sensitivity=100%

3. Patients with other viral infections:

		Anti-HAV IgG ELISA Test		
		negative	positive	Total
Comparison assay	Negative	15	0	15
	Positive	0	20	20
	total	0	0	35

Conformity=100%

4. Auto-immune patients:

		Anti-HAV IgG ELISA Test		
		negative	positive	Total

Comparison assay	Negative	4	0	4
	Positive	0	16	16
	total	4	16	20

Conformity=100%

5. Patients with acute hepatitis A

		Anti-HAV IgG ELISA Test		
		negative	positive	Total
Comparison assay	Negative	42	0	42
	Positive	0	9	9
	total	42	9	51

Conformity=100%

6. Hepatitis A patients in convalescent period:

		Anti-HAV IgG ELISA Test		
		negative	positive	Total
Comparison assay	Negative	10	0	10
	Positive	0	18	18
	total	10	18	28

Conformity=100%

7. Hepatitis A carriers:

		Anti-HAV IgG ELISA Test		
		negative	positive	Total
Comparison assay	Negative	85	0	85
	Positive	0	22	22
	total	85	22	107

Conformity=100%

5.11.2. Analytical sensitivity = 0.121 PEI U/ml = 0.157 IU/ml.

Conc. (E/ml)	OD	Log (Conc.)	Log (OD)
1	0.121	0	-0.9172146
0.5	0.207	-0.30103	-0.6840297
0.2	0.499	-0.69897	-0.3018995
0.1	0.77	-1	-0.1135093
0.05	1.125	-1.30103	0.0511525
Cutoff	0.636	-0.91776471	-0.1965429
Sensitivity		0.121	PEI U/ml

5.11.3.	Precision	
	Intra-assay reproducibility: Intra-assay CV% < 20	
	Inter-assay reproducibility: Inter-assay CV% <25	

5.11.4.	Traceability:
	Concentration of Anti-HAV Positive Control = 7 ±4 PEI U/ml = 9.1 ±5.2 IU/ml
5.12.	**Flow Chart of Test Procedure**

THE SIMPLIFIED PROCEDURE SHOULD BE USED ONLY BY EXPERIENCED USERS. NEW USERS ARE ADVISED TO READ AND FOLLOW THE DETAILED TEST PROCEDURE CAREFULLY.

Add 10µl controls (3xNC, 2xPC) and add 10µl of each specimen into wells. Reserve 2 wells for the blanks.

Add 100µl of Anti-HAV Peroxidase solution into each reaction well, except 2 blanks.

> **Incubate the plate at +37±1°C for 1 hour**

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> **Wash the plate**

(Choice one of the following two methods for color development)

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> **Mix equal volumes of TMB Substrate Solution A and B. Add 100µl of the mixed solution to wells.**

> **Add 50µl of TMB Substrate Solution A to wells and then add 50µl of TMB Substrate Solution B. Mix well, gently.**

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> **Incubate at R.T. for 30 minutes.**

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> **Add 100µl of 2N H_2SO_4 into each well.**

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> **Determine absorbance using 450 nm as reading wavelength with 620-690 nm reference wavelength[*7].**

6.	References:

1. Melnick JL. History and epidemiology of Hepatitis A virus. J Infect Dis 1995:171(Suppl 1):2-8

2. Koff RS. Hepatitis A. Lancet 1998:341:1643-49

3. Lemon SM.Binn LN.Serum neutralizing antibody response to hepatitis A virus. J Infect Dis. 1983; 148:1033

4. Duermeyer W.,Van der Veen J., Koster B. "ELISA in Hepatitis A". Lancet 1978; 1 (8068).:823-824

5. Lemon SM. Inactivated hepatitis A virus vaccines. Hepatology.1992:15:1194-1197

6. Craig AS, Schaffer W. Preventation of hepatitis A with the hepatitis A vaccine N Engl J Med 2004: 350: 476-481

7. The reference wavelength of spectrophotometer can be 620nm to 690nm. However, user should validate the photometer in combination with this kit before.