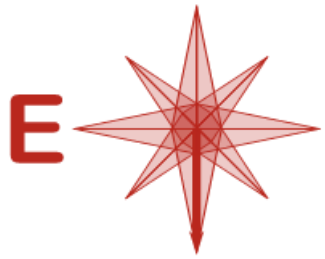
bi-x-act® of FINLAND

Anti-HAV IgM ELISA 96 and 480 Well Plate Test

CE IVD

Productcodes: 2HAVM96
2HAVM480



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1.	**Intended Use**
	Anti-HAV IgM ELISA Test is an enzyme immunoassay kit for in vitro qualitative detection of IgM antibody to hepatitis A virus (Anti-HAV IgM) 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 (1-2). 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). Hepatitis A antigen can be detected in the feces only briefly before or at the onset of infection becoming generally undetectable during the late acute stage (3). The antibody specific to HAV during the acute phase of hepatitis A is the IgM type (Anti-HAV IgM), which decreases then being replaced by IgG type (Anti-HAV IgG) during early and late convalescence (4). Anti-HAV IgM usually disappears 3 to 4 months after the acute phase. An acute hepatitis A virus infection can be assumed if anti-HAV IgM antibody is detected (5). Anti-HAV IgM antibody develops only very rarely after vaccination (6). Assays to detect anti-HAV IgM antibodies are useful in distinguishing hepatitis A infection from other types of infections **Anti-HAV IgM ELISA Test** is a fast test for the qualitative detection of IgM antibody to Hepatitis A virus in serum or plasma (heparin, citrate or EDTA) specimens. This is an enzyme linked immunosorbent assay (ELISA) which utilizes Anti-human IgM on microtiter wells as solid phase and HAV Ag and peroxidase-conjugated Anti-HAV in liquid phase in an "IgM capture" principle to detect Anti-HAV IgM levels in serum or plasma. Specimens with absorbance values **greater** than the Cutoff Value are considered **REACTIVE for Anti-HAV IgM.** Specimens with absorbance values **less or equal** than the Cutoff Value are considered **NONREACTIVE for Anti-HAV IgM.** 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 IgM ELISA Test is a solid-phase enzyme immunoassay (ELISA= enzyme-linked immunosorbent assay) –- based on the principle of "IgM capture". The solid phase of the microtiter plate is made of polystyrene wells coated with anti-human IgM, while peroxidase-conjugated Anti-HAV acts as liquid phase. When a serum or plasma specimen containing Anti-HAV IgM is added to the Anti-human IgM-coated wells and incubated, IgM antibodies present in the specimen bind to the Anti-

human IgM on the wells. After addition of an HAV Ag-containing solution and a solution containing peroxidase-conjugated anti-HAV a further incubation takes place, during which (Anti-h IgM) • (Anti-HAV IgM) •(HAV Ag) • (Anti-HAV• peroxidase) complex is formed on the wells. After washing the microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. If Anti-HAV IgM is present in the specimen, after washing, the activity of peroxidase on the wells reflects the content of anti-HAV IgM in a 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[*8]

The above described test principle is also shown as follows:

A. Specimen (containing antibodies IgM Anti-HAV):
1. Plate (Anti-h IgM) + specimen (containing IgM Anti-HAV)
   → Plate (Anti-h IgM)· IgM Anti-HAV
2. Plate (Anti-h IgM)· IgM Anti-HAV + HAV + Anti-HAV·peroxidase
   → Plate (Anti-h IgM)· IgM Anti-HAV·HAV·(Anti-HAV·HRPO) complex
3. Wash to remove the unbound materials.
4. plate (Anti-h IgM)· IgM Anti-HAV·HAV·(Anti-HAV·HRPO) complex
   + TMB solution → light blue to blue color.
5. Light blue to blue color + 2N H_2SO_4 → light yellow to yellow color, measured at 450 nm with a selected reference wavelength within 620 to 690 nm[*1].

B. Specimen (without antibodies IgM Anti-HAV):
1. Plate (Anti-h IgM) + specimen (without IgM Anti-HAV) → Plate (Anti-h IgM)
2. Plate (Anti-h IgM) + HAV + Anti-HAV· peroxidase → Plate (Anti-h IgM)
   ------ no complex will form
3. Wash to remove the unbound material.
4. Plate (Anti-h IgM) + TMB solution (colorless) → colorless
5. colorless + 2N H_2SO_4 → colorless, measured at 450nm with a selected reference wavelength within 620 to 690 nm[*1].

4. Description of Materials Provided & Product Code System

● **Storage Condition:** Item 1 - 8 on the following reagent table should be refrigerated at + 2 to +8°C . Washing Solution D (20x) and 2N H_2SO_4 can be stored at + 2 to +30°C.

ITEMS	**Components**	**Description**	**Qt. per 96 tests**
(1)	Anti-IgM Coated Plate	Microtiter plate coated with purified antibody to human IgM.	1 plate
(2)	Anti-HAV · Peroxidase Solution	Anti-HAV · peroxidase (horseradish) conjugate in buffer with protein stabilizers. Preservatives: 0.003% gentamycin and 0.01% thimerosal.	1bottle, 7 ml

(3)	Anti-HAV IgM Positive Control	Serum containing diluted Anti-HAV IgM in buffer with protein stabilizers. Preservatives: 0.003% gentamycin and 0.01% thimerosal.	1 bottle, 2 ml
(4)	Specimen Diluent	Protein stabilizer in buffer. Preservatives: 0.003% gentamycin and 0.01% thimerosal.	1 bottle 11 ml
(5)	Hepatitis A Virus Antigen Solution	Hepatitis A virus antigen in buffer and protein stabilizer. Preservatives: 0.003% gentamycin and 0.01% thimerosal.	1 bottle, 7 ml
(6)	Anti-HAV IgM Negative Control	Protein stabilizer in buffer. Preservatives: 0.003% gentamycin and 0.01% thimerosal.	1 bottle, 2 ml
(7)	TMB Substrate Solution A	3,3',5,5'-tetramethylbenzidine (TMB) in an organic base (0.6 mg/ml).	1 bottle, 10 ml
(8)	TMB Substrate Solution B	Citric acid buffer containing 0.03% H_2O_2.	1 bottle, 10 ml
(9)	Conc. Washing Solution D (20x)	Phosphate buffer with tween-20.	1 botlle 52 ml
(10)	2N H_2SO_4	2N sulfuric acid	1 bottle 12 ml

● ACCESSORIES: (provided as needed)

ITEMS	Components
(11)	Adhesive Slips
(12)	Absorbent Pads
(13)	Black Cover

● OTHER MATERIALS AND DEVICES REQUIRED, BUT NOT PROVIDED

ITEMS	Components
(1)	5 µl, 50 µl, 100µl and 1.0 ml micropipettes and tips are needed
(2)	100 ml of 0.15M Normal Saline.
(3)	Incubator or water bath with temperature control at +37 °C.
(4)	Tubes for specimen dilution.
(5)	Plate washing equipment.
(6)	ELISA Microwell Reader: Dual wavelength 450 nm with 620-690 nm as reference wavelength[*1], bandwidth 10nm.
(7)	Purified water: distilled or deionized water.
(8)	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 IgM ELISA Test Kit	+2～+8 °C	Original	15 months
		Once open	1 month
Anti-HAV IgM Positive Control	+2～+8 °C	Original	15 months
		Once open	1 month
Anti-HAV IgM Negative Control	+2～+8 °C	Original	15 months
		Once open	1 month
HAV Antigen Solution	+2～+8 °C	Original	15 months
		Once open	1 month
Specimen Diluent	+2～+8 °C	Original	16 months
		Once open	1 month
Anti-human IgM Plate	+2～+8 °C	Original	15 months
		Once open	2 month
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.	**Instruction 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, HAV solution, 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 your treatment practice of potential bio-hazardous waste or treated as follows: Both liquid and solid waste should be autoclaved maintaining +121°C for at least 30minutes. 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 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.	
WARNING	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 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 immuno-analyzer. 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 two wells for blanks, three wells for Negative Control, two wells for Positive Control, and one well for each specimen. Reserve 2 wells for Blanks. Add **100 µl** of Negative Control to each of three wells, **100 µl** of Positive Control to each of the two wells, and **100 µl** of Specimen Diluent to each of the other reaction wells for the test specimens.
5.5.3.	Make 1+200 dilution of each specimen: Prepare the tubes for dilution as number of specimens. Add 1.0 ml of Saline

	Solution and 5 µl of each specimen to each tube, respectively and shake to mix.
5.5.4.	Add **5 µl of each diluted specimen** to each well containing Specimen Diluent, respectively.
5.5.5.	Gently tap the plate.
5.5.6	Seal the plate with an adhesive slip.
5.5.7.	Incubate the plate in incubator or water bath at +37 ± 1°C for **one hour**.
5.5.8.	At the end of the incubation period, remove and discard the Adhesive Slip and wash plate in accordance with "5.4. **Plate washing procedure**".
5.5.9.	Add **50 µl** of Hepatitis A Virus Antigen Solution and **50 µl** of Anti-HAV·Peroxidase Conjugate Solution into each reaction well except the Blanks. Apply new adhesive slip.
5.5.10.	Incubate the plate in incubator or water bath at +37 ± 1°C for **one hour**.
5.5.11.	At the end of the incubation period, remove and discard the adhesive slip, wash the plate in accordance with "5.4. **Plate washing procedure**".
5.5.12.	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 two blank wells. B. Add 50 µl of TMB Substrate Solution A first, then add 50 µl of TMB Substrate Solution B into each well including the two blanks. Mix well gently.
5.5.13	Cover the plate with black cover and incubate at room temperature for 30 minutes.

5.5.14	Stop the reaction by adding 100 µl of 2N H_2SO_4 to each well including the blank.
5.5.15	Determine the absorbance of controls and test specimens within 15 minutes with a photometer at 450 nm with a selected reference wavelength within 620 to 690 nm[*1]. Use the blank well to blank the photomer. **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 Tested Results**
5.6.1.	Calculation of the NCx (Mean Absorbance of Negative Control). Example: Sample No. Absorbance 1 0.080 2 0.085 3 0.079 NCx = (0.080+0.085 + 0.079) / 3 = 0.081 NCx **must be ≤ 0.2, otherwise, the test is invalid.**
5.6.2.	Calculation of PCx (Mean Absorbance of Positive Control)

	Example: Sample No. Absorbance 1 1.223 2 1.205 PCx = (1.223 + 1.205) /2 = 1.214 PCx **must be ≥0.5 , otherwise, the test is invalid.**
5.6.3.	Calculation of **P-N Value** **P-N =** PCx **-** NCx Example: P - N = 1.214 – 0,081 = 1,133 **P - N Value must be ≥0.3, otherwise, the test is invalid.**
5.6.4.	Calculation of the **Cutoff Value** **Cutoff Value = NC**x **+ (PC**x**)/4** Example: Cutoff Value = 0.081+(1.214/4) = 0.385
5.6.5.	Calculation of the **Retest Range** **Retest Range = Cutoff Value ±10%** Example: Cutoff Value = 0.385 Retest Range = (0.385 - 0.039) to (0.385 +0.039) = 0.346 to 0.424
5.7.	**Validity of Test Runs**
5.7.1.	**NCx must be≤0.2 , otherwise, the test is invalid.**
5.7.2.	**PCx must be ≥0.5 , otherwise, the test is invalid.**
5.7.3.	**P-N Value must be ≥0.3 , otherwise, the test is invalid.**
5.8.	**Interpretation Results**
5.8.1.	Specimens with absorbance values **LOWER** than the **Cutoff Value** are considered **non-reactive** for Anti-HAV IgM
5.8.2.	Specimen with absorbance value **GREATER** than or **EQUAL TO** the **Cutoff Value** is considered **reactive** for Anti-HAV IgM.
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 e**i**ther 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 mg/ml had no effect on the test result. 3. Bilirubin up to 0.3 mg/ml had no effect on the test result. 4. Triglyceride up to 5.0 mg/ml had no effect on the test result. 5. A hheumatoid factor high positive specimen exhibited a false positive result. 6. Pregnancy did not effect the test result.

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

	Anti-HAV IgM ELISA Test			
Comparison assay		negative	positive	Total
	Negative	1378	0	1378
	Positive	4	188	192
	total	1382	188	1570
Diagnostic sensitivity = 100% x 188/192 = 98% Diagnostic specificity = 100% x 1378/1378=100%				

2. Patients with acute hepatitis A:

	Anti-HAV IgM ELISA Test			
Comparison assay		negative	positive	Total
	Negative	51	0	51
	Positive	0	0	0
	total	51	0	51
Conformity = 100 %				

3. Hepatitis A patients in convalescent period:

	Anti-HAV IgM ELISA Test			
Comparison assay		negative	positive	Total
	Negative	28	0	28
	Positive	0	0	0
	total	28	0	28
Conformity = 100 %				

4. Chronic hepatitis A carriers:

	Anti-HAV IgM ELISA Test			
Comparison		negative	positive	Total
	Negative	107	0	107

assay	Positive	0	0	0
	total	107	0	107
Conformity = 100 %				

5. Auto-immune patients:

	Anti-HAV IgM ELISA Test			
Comparison assay		negative	positive	Total
	Negative	20	0	20
	Positive	0	0	0
	total	20	0	20
Conformity = 100%				

6. Patients with HAV infection

	Anti-HAV IgM ELISA Test			
Comparison assay		negative	positive	Total
	Negative	19	0	19
	Positive	0	0	0
	total	19	0	19
Conformity = 100%				

7. Patients with other viral infections:

	Anti-HAV IgM ELISA Test			
Comparison assay		negative	positive	Total
	Negative	35	0	35
	Positive	0	0	0
	total	35	0	35
Diagnostic specificity/Conformity= 100%				

5.11.2. Analytical Sensitivity

Analytical sensitivity ≤ 100 U/ml

e.g. (Lot. No.: 2PT) Analytical sensitivity = 61.7 U/ml

Conc. (U/ml)	OD	Log (Conc.)	Log (OD)
200	1.256	2.30103	0.09898964
100	0.846	2	-0.07262964
50	0.385	1.69897	-0.41453927
25	0.250	1.39794001	-0.60205999
Cutoff	0.506	1.79003187	-0.29584948
Sensitivity **(U/ml)**	61.7	----------	----------

Linear regression	
R	0.991195

		R^2	0.982468
		Adjusted R^2	0.973702
		Standard error	0.051643
		Number of observed value	4

5.11.3.	**Precision**
	Intra-assay reproducibility: Intra-assay CV% < 15
	Inter-assay reproducibility: Inter-assay CV% < 20
5.11.4.	**Traceability:**
	Concentration of Anti-HAV IgM Positive Control = 800±200 U/ml

5.13. Flow chart of the test procedure

Simplified procedure of Anti-HAV IgM ELISA Test

Dilute 5µl of each specimen with 1 ml saline solution. DO NOT DILUTE CONTROLS.

↓

Add 100µl Controls (3xNC, 2xPC) and 100µl specimen diluent into wells. Reserve 2 wells for Blanks.

↓

Add 5µl of the diluted specimen into each of wells containing 100µl specimen diluent respectively.
Seal with adhesive slip.

↓

Incubate at ±37 ±1°C for one hour

↓

Wash the plate

↓

Add 50µl of HAV Antigen Solution and 50µl Anti-HAV·Peroxidase Conjugate Solution into each reaction well, except Blanks.

↓

Incubate at ±37 ±1°C for one hour

↓

Wash the plate

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

/ \

Mix equal volumes of TMB Substrate Solution A and B. Add 100 µl 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.

\ /

Incubate at R.T. for 30 minutes.

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Add 100µl of 2N H_2SO_4 into each well.

↓

Determine absorbance using 450nm as reading wavelength with 620-690 nm reference wavelength[*1].

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**:** 1033-1039.
4. Duermeyer W, Van der Veen J, Koster B. ELISA in Hepatitis A. Lancet. 1978; 1(8068):823-824.
5. Bower WA, Nainan OV, Han X, Margolis HS. Duration of viremia in hepatitis A virus infection. J Infect Dis. 2000;182:12-17.
6. Craig AS, Schaffner W. Prevention of hepatitis A with the hepatitis A vaccine N Engl J Med 2004; 350:476-481
7. The reference wavelength of spectrometer can be 620nm to 690nm. However, user should validate the photometer in combination with this kit before use.