



# ATLAS ANTI-HCV ELISA

(Indirect principle)

## INTENDED USE

ATLAS ANTI-HCV ELISA is an enzyme-linked immunosorbent assay for the qualitative determination of antibody to hepatitis C virus (HCV) in human serum or plasma. It is used for screening blood donors and diagnosing patients related to infection with hepatitis C virus.

## PRINCIPLE OF THE PROCEDURE

ATLAS ANTI-HCV ELISA is based on indirect principle. The microwells are coated with highly purified antigen to HCV. Purified anti-human IgG coupled to horseradish peroxidase (HRP) serves as the conjugate with tetramethylbenzidine (TMB) and peroxide as the substrate.

During the course of the first incubation, any anti-HCV antibodies in the sample will bind to the immobilized antigens. Following washing to remove unbound material, the captured anti-HCV antibodies are incubated with peroxidase conjugated monoclonal anti-human IgG. During the course of the second incubation, the conjugate will bind to antibody immobilized in the first step. After removal of excess conjugate, bound enzyme is detected by the addition of a solution containing tetramethylbenzidine (TMB) and peroxide, color develops which turns yellow when the reaction is stopped with sulfuric acid.

If anti-HCV is present in the sample, an intense color develops. However, when the sample is free of anti-HCV, no or low color forms with the addition of substrate.

## REAGENTS PROVIDED IN THE KIT

For in vitro diagnostic use.

Store all reagents at 2~8°C when not in use.

Expiry date on the kit indicates the date beyond which reagents should not be used.

1. Microwell plate 1 plate  
Blank, white or yellowish microwell strips fixed on white strip holder. The plate is sealed in aluminium pouch with desiccant.  
12×8 wells per plate. Each well contains recombinant antigens to HCV.  
The microwell stripes can be broken to be used separately. Place unused microwell stripes in the plastic sealable bag provided along with the desiccant and return to 2~8°C.
2. Negative control 1 vial  
Blue liquid filled in vial with green screw cap  
Protein stabilized buffer tested non-reactive for HCV antibodies. Preservatives: 0.1% ProClin 300.  
Ready to use as supplied. Once open, stable for one month at 2-8°C.
3. Positive control 1 vial  
Red Liquid filled in vial with red screw cap.  
Anti-HCV antibodies diluted in protein stabilized buffer. Preservatives: 0.1% ProClin 300.  
Ready to use as supplied. Once open, stable for one month at 2-8°C.
4. Specimen diluent 1 vial  
Blue liquid filled in a white vial with blue screw cap.

- Protein-stabilized buffer, casein and sucrose solution.  
Ready to use as supplied. Once open, stable for one month at 2-8°C.
5. HRP-Conjugate Reagent 1 vial  
Red Liquid filled in a white vial with Red screw cap.  
Horseradish peroxidase-conjugated with rabbit anti-human IgG antibodies.  
Ready to use as supply. Once open, stable for one month at 2-8°C.
  6. Stock wash buffer (Dilute before use) 1 bottle  
Colorless liquid.  
PH7.2, 20x PBS (Containing Tween 20 as a detergent)  
The concentrate must be diluted 1:19 with distilled/de-ionized water before use. Once diluted, stable for one week at room temperature or for 2 weeks at 2-8°C.
  7. Chromogen solution A 1 vial  
Colorless liquid filled in white vial with green screw cap.  
Urea peroxide solution.  
Ready to use as supplied. Once open, stable for one month at 2-8°C.
  8. Chromogen solution B 1 vial  
Colorless liquid filled in the black vial with black screw cap.  
TMB solution. Tetramethylbenzidine dissolved in citric acid.  
Ready to use as supplied. Once open, stable for one month at 2-8°C .  
Store it in dark. Avoid light and heat.
  9. Stop solution 1 vial  
Colorless liquid filled in white vial with yellow screw cap.  
Diluted sulfuric acid solution (2.0M H<sub>2</sub>SO<sub>4</sub>).  
Ready to use as supplied.
  10. Plastic sealable bag 1 unit  
Use for enclosing the stripes not in use.
  11. Plate sealer (disposable) 2 sheets  
Clear, papery adhesive sheet to be used to the reactive microplate wells during each incubation to prevent evaporation or contamination of the wells.

#### **ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED BUT NOT PROVIDED**

1. Freshly distilled or de-ionized water.
2. Disposable gloves.
3. Timer.
4. Appropriate waste containers for potentially contaminated materials.
5. Disposable V-shaped troughs.
6. Dispensing system and/or pipette (single or multi channel), disposable pipette tips.
7. Absorbent tissue or clean towel.
8. Dry incubator or water bath, 37± 0.5°C.
9. Microshaker for dissolving and mixing conjugate with samples.
10. Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
11. Microwell aspiration/wash system.

#### **SPICEMEN COLLECTION, TRANSPORT AND STORAGE**

1. Collection: Serum or plasma samples may be used. Blood collected by venipuncture should be allowed to clot naturally .Care should be taken to ensure that the serum samples are fully clotted. Any visible particulate matter in the sample should be removed by centrifugation. Plasma samples collected into EDTA, sodium citrate or heparin may be tested. Hemolyzed samples should not be

used as they can give false results in the assay. Do not heat inactivate the sample since this can cause sample deterioration.

2. **Transport and Storage:** Store samples at 2-8°C. Samples not required for assay within 3 days should be stored frozen (-20°C or lower). Avoid multiple freeze-thaw cycles.

## **ASSAY PROCEDURE**

Make sure all the reagents are in the period of validity and all reagents are the same lot.

Bring all reagents to room temperature (15 to 30°C) before use (appropriately 30 minutes).

1. **Preparing Wash buffer:**

Check the wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve.

Dilute the stock wash buffer at 1: 20 with distilled water or deionized water.

Mix well before use.

2. **Numbering Microwells:**

Set the strips needed in strip-holder, and number sufficient wells including 3 negative controls, 2 positive controls, and 1 blank.

**Note: Use a separate disposal pipette tip for each specimen and Control in order to avoid cross-contamination**

3. **Adding Specimens / controls:**

Add 100µl specimen diluent into each well except the blank. Dispense 10µl specimen, 10µl negative control and 10µl positive control into their respective wells. Mix gently.

4. **Incubating:**

Place a plate sealer on the plate. Incubate the plate for 30 min at 37°C.

5. **Washing:**

Remove and discard the plate sealer. Aspirate the liquid and rinse each well 5 times with the prepared wash buffer. It is necessary to soak the microwells 30~60 seconds each time. Make sure that each microwell is full of the prepared wash buffer per washing. After rinsing, turn the strips down onto blotting paper or clean towel, and tap the plate to remove any remainder.

6. **Adding HRP-Conjugated Reagent:**

Dispense 100µl HRP-Conjugate Reagent into each well except the blank. Mix gently.

7. **Incubating:**

Place a plate sealer on the plate. Incubate the plate for 30 min at 37°C.

8. **Washing:**

The same procedure as step 5.

9. **Coloring:**

Dispense 50µl Chromogen solution A and 50µl Chromogen solution B respectively into all wells, mix gently by tapping the plate. Place a plate sealer on the plate and put it at 37°C for 15 minutes protected from light.

10. **Stopping reaction**

Remove and discard the plate sealer. Using a multichannel pipettor, add 50µl stop solution into each well and mix gently by tapping the plate.

11. **Measuring the absorbance:**

Determine the absorbance for each well at 450nm. If a dual filter instrument is used, the reference wavelength should be 630nm.

### **Note:**

1. Do not touch the bottom exterior surface of the wells. Fingerprints or scratches may interfere with

- microwell reading.
- Shake the reagents gently before use.
  - The prepared diluted wash buffer should be stored at room temperature less than 7 days. It is necessary to store it at 2~8°C for longer time and note the date of production and expiration.
  - Calibrate the pipette frequently to assure the accuracy. Use a separate disposal pipette tip for each specimen and different reagents in order to avoid cross-contamination.
  - It's necessary to assure the temperature is 37°C during incubation. It is suggested to use water tank and the microwell plate should float on water. If using dry box, do not open the door frequently.
  - Avoid touching bleaching powder or disinfectant in the procedure, it will affect the result of the assay.

## INTERPRETATION OF RESULTS

Each microplate must be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed.

Normally, the individual absorbance value of negative control is lower than or equal 0.08, while of positive control is higher than or equal 0.50. The values of the negative control will be used for Cut-off calculation and negative / positive interpretation. The values of the positive control will show whether it is a successful assay. If either value of the positive controls is lower than 0.50, the run is invalid and should be retested.

### 1. Calculation of cut-off value

$N_c$  = the mean absorbance value for three negative controls.

**(If the mean value is lower than 0.02, take it as 0.02.)**

Cut-off value (C.O) =  $N_c + 0.12$

### 2. Interpretation

O.D. value of the specimen = S

**Positive:  $S/C.O \geq 1$**

**Negative:  $S/C.O < 1$**

**Note:** If the sample  $O.D. \leq Cutoff \times 2$ , sample should be retested. If it is the repeatable reactive, the conclusion will be positive. If the result of retest differs from the former result, sample and test again after ten days.

Example :

### 1. calculation of $N_c$ :

Well No.	Absorbance
C5	0.020
D5	0.016
E5	0.018
<hr/>	
Total	0.054

$$N_c = 0.054 / 3 = 0.018$$

□ The mean value is lower than 0.02, so take it as 0.02.

### 2. calculation of Cut-off (C.O.)

$$C.O. = 0.02 + 0.12 = 0.14$$

### 3. Some data from Lab-testing:

Positive control	Negative sample 1	Negative sample 2	Negative sample 3	Weak positive 1	Weak positive 2	Strong positive 1	Strong positive 2
2.345	0.015	0.012	0.026	0.657	0.764	1.864	2.514

## **SAFETY PRECAUTIONS**

1. Do not eat, drink, smoke or apply cosmetics in the assay laboratory.
2. Do not pipette solutions by mouth.
3. Avoid direct contact with all potentially infectious materials by using protective clothing such as lab coats, protective glasses and disposable gloves. Wash hands thoroughly at the end of each assay.
4. All samples, biological reagents used in the assay should be considered as potentially infectious materials. They should therefore be disposed off in accordance with established safety procedures.
5. The stop solution (2M H<sub>2</sub>SO<sub>4</sub>) is a strong acid. Wipe up spills immediately.

## **STORAGE INSTRUCTION**

1. Store at 2-8°C
2. Valid for 12 months from the date of manufacturing.

## **REFERENCES**

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