

HCV Ab

3rd Generation enzyme immunoassay for the determination of antibodies to Hepatitis C Virus (HCV) in human serum or plasma.
For professional *in vitro* diagnostic use only - 96 tests

Catalogue No 7/032A

INTRODUCTION

HCV is the aetiological agent responsible for the disease known as Non-A, Non-B Hepatitis, or Hepatitis C. The clinical symptoms of Hepatitis C are jaundice, acute hepatitis, and elevated liver enzymes. Roughly half of the individuals who contact the disease develop chronic hepatitis, and 20% develop liver cirrhosis and an increased risk of developing liver cancer. The virus is largely transmitted via contaminated blood products by blood transfusion, haemodialysis and intravenous drug use.

This immunoassay employs both synthetic and recombinant HCV peptides for the detection of antibodies to HCV in human serum or plasma. These peptides correspond to the conserved epitopes of the virus.

Specimens with absorbances greater than or equal to the cutoff value are defined as initially reactive. Initially reactive specimens **MUST BE** re-tested to exclude the possibility of a cross-reaction. Specimens, which are reactive in a duplicate test, are defined as repeatable reactive. In diagnosing HCV such specimens must be confirmed as HCV positive by using a western blot test. Laboratories should follow the guidelines of their own national public health ministry.

Intended Use

1. For screening of blood donors.
2. For monitoring individuals with a higher than normal risk of contracting hepatitis, e.g. patients, technicians or nursing personnel in renal dialysis units or clinical laboratories.
3. As an aid in the diagnosis of liver disease.

Principle of the Method

Microplates are coated with HCV specific synthetic antigens derived from "core" and "ns" regions encoding for conservative immunodominant antigenic determinants (core, NS₃, NS₄ and NS₅).

The solid phase is first treated with the diluted sample and HCV Ab are captured, if present, by the antigens. After washing out all other components of the sample, in the 2nd incubation bound HCV Ab are detected by the addition of anti HlgG antibody, labeled with peroxidase (HRP).

The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti-HCV antibodies present in the sample.

PRODUCT CONTENTS

For 96 test assay

1. **Coated Microplate:** 12 x 8 well strips.
Each microplate well is coated with HCV specific antigen, and sealed in an aluminium bag containing silica gel as desiccant.
2. **Negative control:** 1 vial of 1.0ml.
Human serum non-reactive for HBsAg, HCV Ab and HIV Ab. Contains sodium azide as preservative. Ready to use, do not dilute.
3. **Positive control:** 1 vial of 1.0ml.
Inactivated human serum with high titre antibodies to HCV. Non-reactive for HBsAg, and HIV. Contains sodium azide as a preservative. Ready to use, do not dilute.

4. **Specimen Diluent:** 1 vial of 20ml.
Phosphate buffered saline with Tween-20 containing heat-treated normal goat serum and gelatin. Contains sodium azide as a preservative.
5. **Conjugate:** 2 vials of 7.5ml.
Phosphate buffered saline with Tween-20 containing normal goat serum, protein stabiliser and goat anti-human IgG horseradish peroxidase conjugate. Contains thimerosal as a preservative.
6. **Wash Solution:** 1 vial of 80ml.
25x concentrate. Contains tris buffered saline with Tween-20.
7. **Chromogen A:** 1 vial of 8ml.
Contains hydrogen peroxide.
8. **Chromogen B:** 1 vial of 8ml.
Contains tetramethylbenzidine.
9. **Stopping Solution:** 1 bottle of 7ml.
Contains 2M sulphuric solution. **Warning** Corrosive, refer also to 'Warnings & Precautions' no. 20.
10. **Plate covers:** 4 pieces
Cover microplate during incubation.
11. **Instructions for use:** 1 copy.

ITEMS REQUIRED BUT NOT PROVIDED

1. Double distilled water.
2. Manual or automatic pipettes capable of delivering 10µl, 50µl, 100µl and 200µl.
3. Pipette tips.
4. Timer.
5. Microplate mixer.
6. Incubator (37°C).
7. An automatic microplate washer.
8. Microplate reader (equipped with a 450nm and 630nm filter).
9. Gloves.

STORAGE AND SHELF LIFE

1. Store the kit and its components at 2-8°C when not in use.
2. Store wash solution concentrate at 2-8°C. If wash solution concentrate has been stored for a long time, it may be appear turbid. Performance will not be affected.
3. Do not remove microplate from storage bag until needed. Opened, unused strips must be stored with the desiccant provided at 2-8°C in the closed pouch.

WARNINGS & PRECAUTIONS

1. Upon receipt, store the kit at 2-8°C.
2. All the reagents are for professional *in vitro* diagnostic use only.
3. Do not use the kit or reagents beyond the expiry date specified on the labels or if damaged.
4. Do not mix reagents from different lots. Controls, conjugate and micro-plates are matched for optimal performance. Use only reagents supplied by the manufacturer and distilled water where instructed.
5. Procedures should be performed carefully in order to obtain reliable results and interpretation.
6. Allow all reagents to reach room temperature before use.



7. The specimen diluent will change colours when serum or plasma added.
8. Changes in the physical appearance of the reagents supplied may indicate deterioration of these materials. Check that the reagent solutions are clear. Any cloudiness or odour is indicative of contamination. Do not use reagents, which are visibly turbid. Except for specimen diluent and wash solution concentrate.
9. Avoid cross-contamination between serum specimens and between reagents when removing them from the vials. Automatic pipettes with disposable tips are recommended. To avoid cross-contamination when dispensing reagents, do not touch the side of the wells with tips. Avoid cross-contamination between conjugate and substrate.
10. For the washing step, use only the Washing Solution provided in the kit. Carefully follow the instructions. Incomplete washing will reduce the performance of this kit.
11. Follow the installation, operation, calibration and maintenance instructions provided by the instrument manufacturers for both the micro-plate reader and micro-plate washer. All the instruments must be well maintained and serviced regularly.
12. Avoid contact between the substrate/chromogen and oxidising agents or metallic surfaces, since this may give rise to unwanted colour formation. Avoid intense light exposure during incubation or reagent preparation. The use of plastic disposable (sterile) containers is recommended for the preparation of chromogen/substrate.
13. Samples and materials potentially infected must be handled with care. All objects which are in direct contact with samples and all residuals of the assay should be treated as potentially infected.
14. Treat all specimens and kit reagents as potentially infectious. There is no test method available that can offer complete assurance that Hepatitis B virus, HIV or other infectious agents are absent. Therefore all human serum specimens and kit reagents should be handled at Biosafety Level 2.
15. Dispose of all specimens and materials used to perform the test as potentially infectious agents. The preferred method of disposal is autoclaving for half an hour at 121°C or above. Disposable materials may be incinerated. Liquid wastes not containing acid may be mixed with sodium hypochlorite in volumes so that the final mixture contains 10% sodium hypochlorite. Allow 30 minutes for decontamination to be completed. Liquid waste containing acid must be neutralised with a proportional amount of base prior to the addition of sodium hypochlorite.
16. Spills should be cleaned up thoroughly using a 5.25% sodium hypochlorite solution.
17. Avoid contact of TMB (chromogen) and SULPHURIC ACID (stop solution) with the skin and mucous membranes. If these reagents come into contact with the skin wash with copious amounts of water. Consult physician immediately.
18. Use protective talc-free gloves, safety spectacles and laboratory coat when working with samples. Never pipette by mouth.
19. Some reagents of the kit contain sodium azide, which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water. Please refer to your local requirements for other waste management.
20. The stopping solution contains 2M sulphuric acid. This reagent is corrosive and carries the following risk and safety phrases: **R35** causes severe burns. **S26** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. **S30** never add water to this product. **S45** in case of accident or if you feel unwell seek medical advice immediately (show the label where possible).
21. Always follow standard laboratory safety procedures for biohazard containment and report any incident or accident according to local Health and Safety Guidelines. The use of reagents according to Good Laboratory Practice (GLP) is recommended.

SPECIMEN PREPARATION

This immunoassay may be performed on human serum, or plasma. Specimens containing precipitates or particulate matter may give inconsistent test results. If necessary, specimens should be clarified by centrifugation prior to testing.

Specimens **MUST NOT** be heat inactivated prior to assay. This immunoassay is not affected by haemolysis of specimens. No adverse effects have been noted in the presence of the anticoagulants EDTA, sodium citrate, or heparin.

PREPARATION OF REAGENTS

1. WORKING SUBSTRATE SOLUTION:

Must be prepared just prior to use. Once prepared, solution is stable for 30 minutes at room temperature. Mix equal volumes of chromogen A and chromogen B thoroughly in a clean bottle before use. Use immediately after mixing.

If small volumes are required, pipette equal volume of chromogen A and chromogen B separately into a clean bottle and mix it. For example, if you require 4ml this solution, pipette 2ml of chromogen A and 2ml of chromogen B separately into a clean bottle then mix completely.

The substrate should be colourless for proper performance of the assay. Any other colour may indicate deterioration of the substance and/or TMB.

2. DILUTED WASH SOLUTION CONCENTRATE:

Dilute 1 volume of wash solution concentrate with 24 volumes of double distilled water. Mix well.

PROCEDURE

1. Open the aluminium bag and take out microplate with the required number of strips. Place leftover strips in the plastic pouch along with the silica gel bag (desiccant) and seal (see 'Storage and Shelf Life'). During the test, the strips must stay in the microplate.
2. Pipette 100µl of specimen diluent to the wells (leave 5 wells for controls and blank). Pipette 100µl of positive control into each of the two wells, and 100µl of negative control into each of the two wells, and pipette 100µl specimen diluent into the remaining well as a blank.
3. Using a pipette, introduce 10µl of specimen to the assigned wells. (Do not add specimen to the blank well).
4. Seal and incubate for 30 minutes at 37°C.

5. Wash the microplate 5 times with the wash solution provided. Use 300µl/well/wash.
6. Blot dry by pressing plate onto absorbent tissue.
7. Add 100µl of conjugate to all wells of the microplate.
8. Seal and incubate for 30 minutes at 37°C.
9. Prepare working substrate solution as described in 'Preparation of Reagents'.
10. Repeat the wash procedure as per steps 5 and 6.
11. Add 100µl of working substrate solution to each micro-well.
12. Seal and incubate for 10 minutes at 37°C.
13. Add 50µl of stopping solution to each micro-well, mix gently.
14. Read the absorbance at 450nm. If a dual filter instrument is used, the reference wavelength should be 620nm or 630nm. NOTE: Absorbance should be read within 30 minutes of the addition of stopping solution to microplate.

Calculation of cut-off value:

The cut-off (CO) value is:

$$CO = P \times 10\% + N$$

If P is greater than or equal 2.500, let P equal 2.500.

Test result:

A test is positive if $S \geq$ cut-off value

A test is negative if $S <$ cut-off value

Calculation Example

$$P=1.960 \quad N=0.012$$

$$\text{Cut-off Value} = P \times 10\% + N$$

$$= 1.960 \times 10\% + 0.012 = 0.208$$

Quality Control

A test-run is only valid if $N < 0.050$ and $P > 0.800$.

Interpretation of Results

1. Specimens with absorbance values less than the cut-off value are considered not reactive by the criteria of this immunoassay, and may be considered negative for antibodies to HCV. Further testing is not required.
2. Specimens with absorbance values greater than or equal to the cut-off are considered reactive. These specimens (using the original sample) should be re-tested in duplicate before final confirmation of the result.
3. Initially reactive specimens, which do not react in either of the duplicates, repeat tests are considered negative for antibodies to HCV. Further testing is not required.
4. Specimens which have been found repeatable reactive are interpreted to be positive for the presence of antibodies to HCV. In most settings it is appropriate to investigate repeatable reactive specimens by additional more specific test.

Assay Schematic

REAGENTS	BLANK	CONTROLS*	SPECIMEN
Specimen Diluent	100µl	-	100µl
Controls	-	100µl	-
Specimen	-	-	10µl
Cover strips with plate sealer			
Incubate for 30 min. at +37°C.			
Dilute the concentrated washing solution 1:25.			
Remove the plate sealer and aspirate the reaction solution from all wells into a biohazard container.			
Wash 5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid. After the last wash, blot the inverted plate on absorbent paper towels.			
Conjugate	100 µl	100 µl	100 µl
Cover strips with a new plate sealer.			
Incubate for 30 min. at 37°C			
Prepare working substrate solution (see 'Preparation of Reagents')			
Repeat washing procedure (as above)			
Working substrate soln.	100 µl	100 µl	100 µl
Cover strips with plate sealer			
Incubate for 10 minutes at 37°C			
Remove the plate sealer			
Stopping solution	50 µl	50 µl	50 µl
Read the absorbance of each well at 450 nm (using 620 or 630 nm as a reference), within 30 minutes of adding the stopping solution.			

*Refer also to 'Procedure' no. 2.

INTERPRETATION

Results

Before interpretation, subtract the value of the blank from all absorbance values, for both the controls and the specimens. The presence or absence of antibody specific for HCV is determined by relating the absorbance of the specimens to the cut-off value.

Abbreviations

N = the mean absorbance of the negative controls

P = the mean absorbance of the positive controls

S = the absorbance of the test specimen