




## HCV Antibody ELISA KIT

**IVD** For In Vitro Diagnostic and professional Use Only

2°C  8°C  
Store at 2-8°C

 96 Tests

### SUMMARY AND EXPLANATION OF THE TEST

Hepatitis C virus (HCV), which was formerly described as the parenterally transmitted form of non-A, non-B hepatitis (NANBH), becomes a chronic disease in 50% of the cases.

HCV can also be transmitted through intravenous drug abuse, sexual, and household contact.

Hepatitis C virus is a single stranded RNA virus with some structural relation to the flavivirus family. Nucleic acid sequences of HCV cDNA clones provided the basis for the construction of recombinant peptides representing putative hepatitis C virus proteins. Anti-hepatitis C virus antibody screening of blood using synthetic or recombinant proteins, helped to identify apparently healthy blood donors with anti-HCV antibodies which otherwise might have transmitted the virus. This is an enzyme linked immunosorbent assay using recombinant proteins derived from core regions of HCV virus to detect the presence of HCV antibodies in human sera.

### PRINCIPLE OF THE ASSAY

This assay is based upon the two- step indirect method. In the first step, sample and recombinant HCV coated microwells are combined. During the incubation, the HCV antibodies present in sample binds to the antigen coated on the wells. After the washing, in the second step, enzyme conjugate is added to the reaction mixture during incubation, the HCV antibodies present in the sample reacts with mouse Anti-human IgG within enzyme conjugate. Then a complex is generated between the solid phase, the HCV Abs within the sample and mouse Anti-human IgG in the enzyme conjugate by immunological reactions. After a second washing, substrate A and substrate B are then added and catalyzed by this complex, resulting in achromogenic reaction. The resulting chromogenic reaction is measured as absorbance. The color

intensity is proportional to the amount of HCV antibodies in the sample.

### MATERIAL PROVIDED

1. **Coated microplates:** One microplate with 96 wells coated with HCV recombinant proteins. Store at 2-8° C.
2. **Enzyme Conjugate:** One red cap vial (11.5 ml) of HRP (horse radish peroxidase) labeled mouse anti-human IgG in a stabilizing buffer containing proteins of bovine origin .contains 0.05 %Proclin 300 preservative . Store at 2-8° C.
3. **Negative Control:** One green cap vial (1.0 ml) of phosphate buffered solution containing proteins of bovine origin.Store at 2-8° C.
4. **Positive Control:** One red cap vial (1.0 ml) of Phosphate buffered solution containing pooled heat-inactivated human serum and plasma positive for HCV antibodies and proteins of bovine origin contains 0.05% Proclin 300 preservative .Store at 2-8° C.
5. **Sample Diluent:** One white cap vial (11.5 ml) of Tris-NaCl buffer and casein. Contains 0.02% sodium azide preservative. Store at 2-8° C.
6. **Substrate Solution A:** One blue cap vial (7.5 ml) of hydrogen peroxide .Store at 2-8° C.
7. **Substrate Solution B:** One brown cap vial (7.5 ml) of 3,3',5,5'-Tetramethylbenzidine (TMB) in buffer solution .Store at 2-8° C.
8. **Stop Solution:** One yellow cap vial (7.5 ml) containing 0.62 mol/l H<sub>2</sub>SO<sub>4</sub>. Ready to use.
9. **Washing solution (20X Conc.):** One transparent cap vial containing 50 ml of 20 times working strength PBS-Tween wash buffer.
10. **Two Plate sealers.**
11. **IFU, One copy.**
12. **One Zip-lock bag.**

### MATERIALS REQUIRED BUT NOT SUPPLIED

1. Precision Micropipettes and tips
2. Incubator (+37° C)
3. Automatic plate washer
4. Microtiter plate reader for the measurement of absorbance at 450 nm.
5. Absorbent pad or paper.
6. Distilled water.
7. Vortex mixer.
8. Magnetic stirrer.
9. Plate shaker.
10. Disposable reagent troughs.
11. Micropipettes and multichannel micropipettes.

### PRECAUTION FOR USERS

1. For in-vitro diagnostic use only.
2. Must not use kit beyond the expiration date.
3. Do not mix components from kits with different lot number.
4. The positive control contains human sourced components, which have been tested and found reactive for HCV antibodies, non-reactive for HIV 1 and HIV 2 Antibodies , Anti-HTLV 1 and 2 , HBsAg and Syphilis . It recommended that all human sources considered potentially infectious .This assay contains materials of animal origin .Bovine components originate from countries where BPS has not been reported.
5. Avoid microbial contamination of reagents.
6. Do not pipette reagent by mouth and no smoking or eating while performing assays.
7. Wear gloves during the whole process and avoid reagents or specimen spilling-out.
8. Wipe up the spills using 5% hypochlorite solution.
9. Decontaminate all liquids or solid wastes before depositing.
10. Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate.
11. When handling conjugate vials, change gloves that have contacted human plasma/sera, since introduction of human IgG/IgM will result in a neutralized conjugate.
12. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.

### STORAGE

1. Store all components at 2-8 ° C. Do not freeze. Avoid strong light.
2. Place unused wells in the Zip-lock bag with desiccant provided, then seal the Zip-lock bag in the aluminum foiled pouch with a plate lid and return to 2-8 °C, under which conditions the wells will remain stable for 2 months, or until the labeled expiry date, whichever is earlier.
3. Seal and return all the other unused reagents to 2-8 ° C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

### SAMPLES COLLECTION

1. Either serum or plasma can be used with this diagnostic kit.
2. Samples collected in tubes containing EDTA, heparin or sodium citrate have no notable interference to this assay.
3. Do not use heat-inactivated samples. Do not use sodium azide

preservative in samples.

- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
- Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
- Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
- Avoid grossly hemolytic, lipemic or turbid samples.
- Cap and store the samples at 18-25 °C for no more than 8 hours, for longer use samples should be capped and stored at 2-8 °C up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 °C. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
- Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
- Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
- If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
- When evaluating serial samples, use the same type of sample throughout the study.
- Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual patient samples.
- This assay was designed and validated for use with human serum or plasma from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.

#### REAGENT PREPARATION

- Bring all reagents to room temperature (18-25 °C) prior to use for at least 30 minutes. Mix all reagents through gently inverting prior to use. Do not induce foaming.
- Adjust the incubator to 37 °C.

- Add 1 volume of wash solution concentrate to 19 volumes of distilled water to give the required volume, and mix well with a magnetic stirrer. The wash solution is stable at room temperature for 2 months.

#### PROCEDURE

- Use only the number of wells required and format the microplates wells for each control and sample to be assayed.
- Reserve one well for blank, add **100 ul of the Negative Control** to each of three wells, **100 ul of the Positive Control** to each of two wells and **100 ul of sample diluent** to the other wells, then Pipette **10 ul of sample** to sample diluent wells. According to the following scheme  
1 A : Blank  
2 A,3 A,4 A : Negative Control Serum  
5 A, 6 A : Positive Control Serum  
7 A : Samples

**Notes: use an individual tip for each pipetting to avoid cross contaminations.**

- Shake on a plate shaker for 30 seconds to completely mix the liquid within the wells.
- Cover the plate with a lid and incubate at 37 °C for 30 minutes.
- Add **350 µl of wash solution**, decant (tap and blot) or aspirate. Repeat 5 additional times for a total of 6 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
- Add **100 µl of enzyme conjugate** to each well except the blank well.
- Cover the plate with a lid and incubate at 37 °C for 30 minutes.
- Add **350 µl of wash solution**, decant (tap and blot) or aspirate. Repeat 5 additional times for a total of 6 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
- Add **50 µl of substrate A**, and then add **50 µl of substrate B** to each well, including the blank well.
- Gently mix for 15 seconds and incubate at 37 °C in the dark for 10 minutes without shaking.
- Add 50 µl of stop solution to each well, including the blank well.
- Gently mix for 15 seconds. It is very important to make sure that the blue color changes to yellow completely.
- Read the absorbance within 20 minutes at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfections) in a microplate reader. Alternatively, the actual absorbance can be

obtained by subtracting the absorbance of each well at 450 nm with the absorbance of the blank well at 450 nm.

#### MEASUREMENT OF RESULTS

##### 1. Negative Control

Calculate the mean absorbance of the replicates of the negative control.

##### 2. Cut-off Value

Calculate the cut-off value by adding 0.1 to the negative control replicates (in case mean absorbance of negative control replicates <0.05, (use 0.05 instead of actual mean).

##### 3. Example

Negative control absorbance: well 1=0.04, well 2=0.023, well 3 =0.015

Mean Negative Control = (0.04 +0.023 +0.015)/3 =0.026

Cut-off Value = 0.1+0.05 =0.15.

#### QUALITY CONTROL

The recommended control requirement for this assay is using positive and negative controls to verify assay performance. The result is valid if the following criteria for controls are both met:

##### 1. **Negative Control:**

Mean absorbance of negative control is lower than 0.1.

##### 2. **Positive Control**

Mean absorbance of positive controls is equal to higher than 0.6.

#### INTERPRETATION OF RESULTS

##### **Nonreactive**

Samples giving an absorbance less than the cut-off value are considered nonreactive.

##### **Reactive**

Samples giving an absorbance equal to or greater than cut-off value are considered initially reactive, which indicate that antibodies to hepatitis C virus have probably been detected using ELISA Kit. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for antibodies to HCV and therefore the patient is probably infected with hepatitis C virus. Blood unit positive for HCV antibodies should be immediately discarded.

#### LIMITATIONS OF THE ASSAY

- This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
- If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.

3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.

#### PERFORMANCE CHARACTERISTICS

##### PRECISION

This assay is designed to have a within-run precision of <10%.

2 human plasma based panel members (1 and 2) were assayed, using 1 batch of reagents, in replicates of 20. Data from this study are summarized in the following table.

Panel Member	Batch	n	Mean	Within-run Precision	
				SD	%CV
1	1	20	1.056	0.058	6.8
2	1	20	0.323	0.024	7.4

This assay is designed to have a between-batch precision of < 15%.

2 human plasma based panel members (1 and 2) were assayed, in replicates of 8, across 3 batches of reagents. Data from this study are summarized in the following table.

Panel Member	n	Mean	Between-Batch Precision	
			SD	%CV
1	24	1.763	0.156	8.9
2	24	0.240	0.023	9.5

##### SENSITIVITY

The diagnostic sensitivity of the product was determined by testing a panel of 100 positive samples, all samples resulted positive; therefore the diagnostic sensitivity was 100%.

Samples	Tested samples	Positive samples
Anti-HCV positive	100	100

##### SPECIFICITY

The diagnostic specificity was determined by testing a panel of 400 negative samples, in parallel with tests already available on the market. The

results obtained show a diagnostic specificity of 99.5%.

		This assay		
		Positive	Negative	Total
Reference test	Positive	100	0	100
	Negative	2	398	400
	Total	102	398	500

#### REFERENCES

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	Catalogue Number		Temperature limit
	In Vitro diagnostic medical device		Caution
	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry