ANTI-HCV ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIAHCV1	Anti-HCV ELISA	96 Tests
EIAHCV2	Anti-HCV ELISA	480 Tests

Intended Use:

Anti-HCV ELISA is an enzyme linked immunosorbent assay (ELISA) for the qualitative detection of antibodies to Hepatitis C Virus in human serum and plasma. It is intended for screening of blood donors and for diagnosing of patients suspected of infection with Hepatitis C virus. This reagent is for In vitro diagnostic use only.

Summary and Principle:

Hepatitis C virus (HCV) is an envelope, single stranded positive sense RNA (9.5 kb) virus belonging to the family of Flaviviridae. Six major genotypes and series of subtypes of HCV have been identified. Isolated in 1989, HCV is now recognized as the major cause for transfusion associated non-A, non-B hepatitis. The disease is characterized with acute and chronic form although more than 50% of the infected individuals develop severe, life threatening chronic hepatitis with liver cirrhosis and hepatocellular carcinomas. Since the introduction in 1990 of anti-HCV screening of blood donations, the incidence of this infection in transfusion recipients has been significantly reduced. The first generation of HCV ELISAs showed limited sensitivity and specificity and was produced using recombinant proteins complementary to the NS4 (c100-3) region of the HCV genome as antigens. Second generation tests, which included recombinant / synthetic antigens from the Core (c22) and nonstructural regions NS3 (c33c, c100-3) and NS4 (c100-3, c200) resulted in a remarked improvement in sensitivity and specificity. The third generation tests include antigens from the NS5 region of the viral genome in addition to NS3 (c200), NS4 (c200) and the Core (c22). Third generation tests have improved sensitivity and shorten the time between infection with HCV and the appearance of detectable antibodies (window period) to 60 days. Anti-HCV ELISA is based on double antigen "sandwich" principle ELISA. This novel for the testing of HCV antibodies method allows detection of very early antibodies including IgM, and IgA in addition to the IgG which is the main target for detection of the previous generation assays. In addition, the method minimizes the unspecific reaction showed by the other methods and thus its utilization increases the specificity in detection.

The Anti-HCV ELISA is solid phase, indirect ELISA method for detection of antibodies to HCV. Microwells are coated with recombinant antigens corresponding to the core and the non-structural regions of the Hepatitis C virus particle. Serum or plasma samples are added to the microwells along with diluent. During the first incubation any anti-HCV antibodies present in the samples will interact with the coated antigens and be immobilised in the wells. The wells are washed to remove sample proteins then anti-human IgG antibody conjugated to horseradish peroxidase (HRP) is added. During the second incubation, the labelled anti-human IgG antibody will be bound the immunocomplexes formed during the first incubation. Unbound Conjugate is removed by another washing step. Chromogen solutions containing tetramethyl-benzidine (TMB) and urea peroxide are added to the wells where the HRP on the immunocomplexes catalyses the hydrolysis of the chromogens to form a blue coloured product. The blue color turns yellow after stopping the reaction with sulphuric acid. The colour intensity can be measured spectrophotometrically and is proportional to the level of anti-HCV present in the sample.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well) 5x96 (480T)	Each microwell is coated with recombinant HCV antigens. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2 - 8°C. Once open the wells are stable for 1 month at 2 - 8°C.
Negative Control	1x0.2ml 3x0.2ml (480T)	Blue coloured liquid of protein-stabilized buffer tested non-reactive for HCV antibody. Ready to use. Once open stable for 1 month at 2 - 8°C.
Positive Control	1x0.2ml 3x0.2ml (480T)	Red coloured solution of protein-stabilized buffer containing a dilution of HCV antibody. Once open, stable for 1 month at 2 - 8°C.
HRP-Conjugate	1x13ml 5x13ml (480T)	Red coloured liquid. HRP conjugated rabbit anti-human IgG. Once open, stable for one month at 2 - 8°C.
Specimen Diluent	1x13ml 5x13ml (480T)	Green coloured liquid consisting of a serum matrix with casein and sucrose.
Wash Buffer (20X)	1x50ml 2x125ml (480T)	PBS at pH 7.4. 20X concentrate. Once open, stable for one month at 2 - 8°C. The concentrate must be diluted 1/20 with distilled water before use. Once diluted it is stable at room temperature for a week or two weeks at 2 - 8°C.
Chromogen A	1x8ml 1x60ml (480T)	Urea peroxide solution. Ready to use. Once open, stable for one month at 2 - 8°C.
Chromogen B	1x8ml 1x60ml (480T)	TMB Solution. Ready to use. Once open, stable for one month at 2 - 8°C.
Stop Solution	1x8ml 1x60ml (480T)	0.5M Sulphuric acid solution. Ready to use. Once open, stable for 1 month at 2 - 8°C.

Plastic Sealable bag, IFU and Cardboard plate covers.

Materials required but not provided:

Distilled water or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, pipettes, disposable pipette tips, absorbent tissue or clean towel, dry bath incubator or water bath, plate reader – single wavelength 450nm or dual wavelength 450/630nm and microwell aspiration system.

Specimen Collection:

Collect serum and EDTA, sodium citrate or heparin plasma by separation after standard venipuncture technique. For serum, the blood must be allowed to clot fully but be separated as soon as possible to avoid haemolysis of the RBC. Any visible particulate matter in the serum or plasma should be removed by centrifugation. Do not heat inactivate samples.

Samples will be stable for analysis up to 3 days at 2 - 8°C or stored at -20°C.

Do not use samples with high content of haemoglobin, bilirubin or triglyceride as these substances can interfere in the ELISA.

The HCV ELISA assay is used only for testing serum or plasma samples. Do not use for testing cadaver samples, saliva, urine or other body fluids or pooled blood

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2 - 8°C. Do not freeze. Keep all components tightly capped and without any contamination.

Precautions and Safety:

The ELISA assays are time and temperature sensitive. To avoid incorrect results, follow the test procedure exactly as specified.

1. **Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.**
2. **Make sure that all reagents are within the expiry indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels.**
3. **IMPORTANT: Allow the reagents and samples to reach room temperature (15 - 30°C) before use. Shake reagent gently before use. Return at 2 - 8°C immediately after use.**
4. **Do not touch the bottom of the plate; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.**
5. **Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.**
6. **Make sure timing of reagent additions are the same for all wells and for each procedure step.**
7. **Make sure the temperature inside the incubator is 37°C.**
8. **Use different pipette tip for each sample and reagent in order to avoid cross-contamination. Only touch the pipette tip against the side of the well, never the bottom of the well.**
9. **The enzyme activity of the HRP-conjugate is affected by dust and strong chemicals including sodium hypochlorite, acids and alkalis. Do not let these substances contaminate the Conjugate reagent.**
10. **If using fully automated equipment do not cover the plate with a plate cover during incubation. The tapping out of well contents after washing, can also be omitted.**
11. **All samples from human origin should be considered as potentially infectious. Strict adherence to Good Laboratory Practice regulations will ensure personal safety**
12. **WARNING: Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the samples or reagents are completely absent. Therefore, handle reagents and samples with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.**
13. **Pipette tips, vials, well strips and sample containers should be collected and autoclaved for not less than 2 hours at 121oC or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should never be autoclaved. MSDS available upon request. Reagents must be disposed of only in accordance with local or national regulations**
14. **Some reagent components may cause toxicity, irritation, burns or have carcinogenic effects as raw materials. Avoid contact of all reagents with skin and the mucosa but especially the Stop Solution, Substrate reagents and the Wash Buffer.**
15. **The Stop Solution contains sulphuric acid. Use it with appropriate care. Wipe up spills, immediately and wash with water if comes into contact with the skin or the eyes.**
16. **Proclin 300 is used as preservative and can cause a reaction on the skin. Wipe up spills immediately or wash with water if comes into contact with skin or eyes.**
17. **Indicators of deterioration of the reagents: The values of Positive and Negative controls fall out of the quality control range may indicate reagent instability or operator or equipment error. Where quality controls are out of range the results should be considered invalid and the samples must be retested. In case of consistently erroneous results and proven deterioration of the reagents, immediately discard the reagents in use and use a new kit. Contact the local Prestige Diagnostics representative.**

Procedure:

Reagent preparation:

Allow the reagents to reach room temperature (15 - 30°C). Check the wash buffer concentration for the presence of salt crystals. If crystals have formed, re-solubilize by warming at 37°C, until crystals dissolve. Dilute the Wash Buffer (20X), for example by adding 50 ml Wash Buffer concentrate to 950 ml of distilled or deionized water. Alternatively add concentrate to distilled water in the ratio 1/20. All other reagents are ready to use as supplied.

STEP 1

Preparation: Reserve 3 wells for Negative Control, 2 wells for Positive Control and one Blank (e.g. A1 – taking care that neither HRP conjugate nor any samples are added to the blank well). Note: If results are read using a plate reader having dual wavelength (450 / 600-650nm) then a Blank well need not be used. Assign samples to wells.

STEP 2

Addition of diluent: Add 100 µl of Specimen Diluent into each well except the Blank well.

STEP 3

Addition of the sample: Add 10 µl of Positive control, Negative Control and samples into their assigned wells except the Blank. Mix by tapping the side of the plate gently.

STEP 4

Incubation: Cover the plate with the plate cover and incubate for 30 minutes at 37°C.

STEP 5

Washing: At the end of the incubation, remove the plate cover and discard the well contents by decantation or aspiration. Add 350 µl of diluted wash solution to all wells and soak for one minute before discarding the buffer. Repeat 4 more times for a total of 5 washes. Use of an automated microplate strip washer is recommended. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.

STEP 6

Addition of HRP Conjugate: Add 100 µl of HRP Conjugate into each well except the Blank well.

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STEP 7

Incubation: Cover the plate with the plate cover and incubate for 30 minutes at 37°C.

STEP 8

Washing: At the end of the incubation, remove the plate cover and discard the well contents by decantation or aspiration. Add 350 µl of diluted wash solution to all wells and soak for one minute before discarding the buffer. Repeat 4 more times for a total of 5 washes. Use of an automated microplate strip washer is recommended. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.

STEP 9

Addition of the chromogens: Add 50 µl of Chromogen A and 50 µl of Chromogen B into each well including the blank. Incubate the plate at 37°C for 15 minutes. Ensure this incubation is carried out in the dark.

STEP 10

Stopping the Reaction: Add 50 µl of Stop Solution to each well and mix gently until the blue colour changes completely to yellow.

STEP 11

Measurement: Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the cut off value and evaluate the results. (Note: Absorbances must be read within 10 minutes of adding the stop solution).

Instructions for Washing:

To remove the potential for poor washing to cause false positive results and a high background, a 5 step automatic wash cycle is required with 350-400ul of diluted wash buffer used per well per wash.

Ensure the microplate washer is adequately maintained. Ensure the liquid dispensing channels are not blocked or contaminated and that correct volume of wash buffer is dispensed each time into the wells.

If plates have to be washed manually, a process of 5 washing cycles, dispensing 350-400ul/well, allowing 60 second soak and aspirating the liquid 5 times is recommended. If poor results are observed with high background, increase the number of washing cycles and soak time per well.

Capture all liquid waste aspirated and treat with 2.5% sodium hypochlorite for 24 hours before being disposed of in the appropriate way

Calculation of results:

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (Abs) with the cut off value (CO) of the plate. If the cut off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well Abs from the absorbances of the specimens and the controls. . If the results are taken from a dual filter plate reader, do not subtract the Blank well Abs value from the sample and control absorbances, just use the values obtained.

Calculation:

Cut off value (CO) = Nc + 0.12

(Nc = the mean absorbance value for 3 negative controls)

Important: If the mean Abs value of the Negative Controls is less than 0.020 then use 0.02 as the Nc value.

Validation:

Blank well: the absorbance must be <0.080 at 450nm.

Positive Control: the absorbance must be ≥ 0.800 at 450/630nm or at 450nm after blanking.

Negative Control: the absorbance must be <0.100 at 450/630nm or at 450nm after blanking.

If one of the Negative Control absorbances does not match the above criteria, this value should be ignored and a mean value should be calculated using the other two values. If more than one Negative Control absorbance does not meet the criteria, the test is invalid and must be re-tested.

Example:

Blank Value	A1: 0.025	450nm (blanking is required only when reading with a single filter)	
Negative control	0.019	0.018	0.021
Positive control	2.381	2.407	

Calculation of Nc: ((0.019+0.018+0.021)/3) = 0.019

The calculated Nc is less than 0.02, so use 0.02 as the Nc value

Calculation of the cut off: 0.02 + 0.12 = 0.140

Interpretation of the results:

Negative Results: (Abs/CO <1) Specimens giving absorbance less than that of the Cut off value are negative for this assay. This indicates that the sample is non-reactive for HCV antibodies and the patient is probably not infected with HCV. So the blood unit does not contain HCV and could be used for transfusion provided that other infectious diseases markers are also absent.

Positive Results: (Abs/CO ≥ 1) Specimens giving an absorbance equal to or greater than the cut off values are considered initially reactive, indicating that HCV antibodies have been probably detected. All initially reactive samples should be re-tested with the this kit before final interpretation. Repeatedly reactive specimens can be considered positive for HCV antibodies.

Borderline: (Abs/CO = 0.9 – 1.1) Specimens with absorbance to cut off ratio between 0.9 to 1.1 are considered borderline and retesting of these specimens in duplicate is required to confirm the initial results.

Follow up, confirmation and supplementary testing of any positive specimen with other analytical systems such as RIBA , PCR is required. Clinical diagnosis should not be established using a single result.

If, after re-testing of initially reactive samples, both wells show negative results (Abs/CO < 0.9), these samples should be considered negative and the original result must be classified as false positive. False positive results may occur due to several reasons, often associated with, but not limited to inadequate washing step.

If after retesting in duplicate, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive samples should be regarded as positive for HCV and the blood unit must be discarded.

After retesting in duplicate, samples with values close to the Cut-off value should be interpreted with caution and considered as borderline, or uninterpretable at the time of sample taking.

Performance Characteristics:

Specificity:

Clinical Specificity of the Anti-HCV ELISA was assessed using a panel of 2910 blood donor samples. Overall clinical Specificity was determined as 99.55%.

	Number of Samples	-	+	False Positive	Specificity
Blood donors	2910	2897	13	13	99.55%

Sensitivity:

Clinical Sensitivity was determined using 480 Hepatitis C patients of which all 480 were confirmed positive by RIBA 3.0. 480 samples tested positive in the Anti-HCV ELISA, therefore, the overall sensitivity was determined as 100%.

Cross Reactivity:

No cross reactivity was observed when samples positive for HAV, HBV, HIV, CMV and TP were tested in the Anti-HCV ELISA. No interference was observed from samples containing up to 2000 U/ml Rheumatoid Factor.

Limitations:

- Positive results must be confirmed with another available method such as PCR and diagnosis made in conjunction with the other clinical and laboratory information.
- Antibodies may be undetectable during the early stages of the disease. Negative results only indicate that the sample does not contain detectable levels of HCV antibody.
- If, after re-testing of the initially reactive specimens, the assay results are negative, these samples should be considered as non-repeatable and reported as negative.
- Kit failure may result from using kits beyond the expiry date, poor washing procedures, contaminated reagents, improper operation of equipment, sample collection issues or timing errors.
- This kit is intended only for testing of serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled blood.
- This assay is a qualitative assay and the results cannot be used to measure antibody concentrations.

References:

1. Alter HJ. (1978) You will wonder where the yellow went: A 15-year retrospective of posttransfusion hepatitis. In: Moore SB, ed. Transfusion-Transmitted Viral Diseases. Alington, VA. Am. Assoc. Blood Banks, pp. 53-38.
2. Alter HJ., Purcell RH, Holland PV, et al. (1978) Transmissible agent in non-A, non-B hepatitis. Lancet I: 459-463.
3. Choo Q-L,Weiner AJ, Overby LR, Kuo G, Houghton M. (1990) Hepatitis C Virus: the major causative agent of viral non-A, non-B hepatitis. Br Med Bull 46: 423-441.
4. Engvall E, Perlmann P. (1971) Enzyme linked immunosorbent assay (ELISA): qualitative assay of IgG. Immunochemistry 8:871-874.

REF	Catalogue number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by Date
	Manufacturer		

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