

HCV CINNASCREEN

Enzyme Linked ImmunoSorbent Assay for detection of Antibodies to the hepatitis C virus

INSTRUCTION FOR USE

Intended Use

This test system is indirect enzyme immunoassay kit for the detection of Antibodies to Hepatitis C Virus (HCV) in human serum and plasma

1. PRINCIPLE OF THE TEST

The mixture of high purified recombinant polypeptides which simulate HCV Structure protein Core and non-structural proteins NS4 and NS5 are adsorbed On the surface of micro plate wells. Test samples and control sera are incubated In the wells. Antibodies to HCV proteins bind with adsorbed polypeptides forming Immune complexes. After washing off immuno peroxidase conjugate is incubated In the wells and binds with immune complexes. After next washing off bound conjugate Might be visualized using tetramethylbenzidine(TMB)solution with hydrogen peroxide to give blue color after termination of enzymatic reaction with sulfuric acid. The value of optical density of colored solution in the wells measured at 450nm is related to the concentration of antibodies to HCV in the sample. This kit is a completely harmless kit as it contains recombinant polypeptides produced In the E.coli and non-infectious positive control sera. However all testing samples ,fluid .After washing, equipment and tools used during test procedure should be considered as potentially hazardous. The following protective steps should be performed:

- Use latex gloves;
- Do not pipette solutions by mouth;
- Fluids after washing should be treated with 4%chloramin es or 6%hydrogen peroxide solution at 1825c for hours;
- Waste should be accumulated in autoclavable container followed by autoclaving or incineration;
- Equipment and tools used during test procedure should be sterilized by rubbing with 70%ethanol solution .

2 . REAGENTS

1) *concentrated washing solution (25)(solution #)*

Each bottle contain 20ml of colorless solution . Opalescent or clear foaming colorless liquid with possible crystal sediment;

The sediment is easily dispersed in 30minutes at 35 – 37c

2) *sample diluent solution (solution#2)*

Each bottle contain 12ml of red colored solution without any sediment .

3) *conjugate diluent solution (solution#3)*

Each bottle contain 12ml of purple Colored solution.

4) *substrate solution mixed with chromogen (TMB) ready to use.*

Each bottle contain 12ml of clear colorless liquid keep in dark place

5) *stop solution*

Each bottle contain 7 ml of sulfuric acid solution.

6) *positive control serum (c+)*

Each tube contain 500 micro liter of red colored solution

7) *negative control serum (c-)*

Each tube contain 300 micro liter of solution

8) *conjugate solution*

Each tube contain 250 micro liter of green colored solution.

9) *ELISA micro plate*

Each 96well micro plate with HCV recombinant polypeptides coated on the surface of the wells.

3 . MATERIALS REQUIRED BUT NOT PROVIDED

- distilled or deionized water;
- chloramines or hydrogen peroxide for disinfection;
- 8 or 12 channel sample for volumes of 10 – 100 micro liter;
- polypropylene pipette tips for 250 – 1000micro liter,
- desk – top laboratory centrifuge, for preparing plasma samples and clearing sera samples;
- measuring glass or cylinder for 500 ml;
- plastic trays for reagent solutions;
- 37 incubator;
- flask for washing fluids and non used reagent solutions accumulation

4 . PREPARATION OF REAGENTS FOR USE

for one 96-well micro plate

PRIOR TO USE KEEP THE OPENED KIT AT ROOM TEMPERATURE FOR 30 MINUTES.NEVER USE COMPONENTS FROM DIFFERENT BATCHES OR MIX THEM

4.1. Washing solution

Dilute the content of 1 bottle of concentrate washing solution #1 in 480 ml of distilled or deionized water after intensive shaking.

Storage up to 24 hours at 4 – 25 c

NOTE : The crystals of buffer components may appear during the storage of washing solution concentrate. In this case the crystals should be completely dissolved by warming up and incubation at 37c

4.2. Solution #2 for dilution of test samples

Provided ready for use. shake before use

4.3. Conjugate solution

Open the bottle of conjugate and transfer 212 micro liter of the solution to 1ml of conjugate diluent #3 and mix carefully .

Storage up to 15minutes at 4 – 25 c in dark place.

4.4. Chromogen (TMB) solution

Ready to use ,Avoid direct light.

4.5. Positive control (c+)

Provided ready for use.

Storage up to 15minutes at 4 – 25c

Control samples should be reconstituted and used just after adding of tested samples into working plate.

4.6. Negative control (c-)

Use as test samples

4.7. Stop solution (1M sulfuric acid solution)

Provided ready for use.

Storage in closed bottle avoid skin contact.

ATTENTION ! TO AVOID INCORRECT RESULTS THE TEST SAMPLES SHOULD BE STORED IN CONDITIONS EXCLUDING BACTERIAL GROWTH. TEST SAMPLES CONTAINING AGGREGATES SHOULD BE CLARIFIED BY CENTRIFUGATION .TEST SAMPLES SHOULD BE STORED AT 4 – 8 C NOT MORE THAN 72 HOURS .FOR LONGER STORAGE TEST SAMPLES SHOULD BE KEPT AT –20 C . MULTIPLE FREEZING AND THAWING OF TEST SAMPLE IS NOT RECOMMENDED.

ATTENTION ! THE USE OF HAEMOLISED SAMPLES AND SAMPLES WITH HYPERLIPIDAEMIA AND BACTERIAL GROWTH MAY LEAD TO INCORRECT RESULTS .THE SAMPLES SHOULD NOT BE HEAT INACTIVATED SINCE NON SPECIFIC RESULTS MAY OCCUR. USE A NEW PIPETTE TIP FOR EACH TEST SAMPLE OR SOLUTION!

5 . TEST PROCEDURE

5.1. Wash micro plate 1 time with washing solution ,using automatic or manual micro plat washer.

ATTENTION ! 200-250 micro liter of washing solution should be dispensed in each well while washing. ensure that the washer fills, incubates for 15-30 sec, and aspirate off. Carefully remove remaining fluid by tapping the plates on tissue paper prior to next step.

5.2. The order of tested and control sera dispensing:

5.2.1. dispense 50 micro liter of sample diluents solution (shake well before use) into 93 wells of 96 well micro plate. Add 50 micro liter of tested serum or plasma into each well.

Mix carefully by pipetting .Add 100 micro liter of sample diluent into well F1 for conjugate control.

5.2.2. Add 50 micro liter of negative control into each of 3 wells (C1,D1,E1).Add 100 micro liter of positive control into each of 2 wells(A1,B1)

Cover the plate with a sealing tape and incubate 45 min at 37c .

Don't place the plates in pile .

5.3. Aspirate off the contents of wells with washer .wash micro plate 5 times by the washing solution and dry as described in step 5.1.

5.4. Dispense 100 micro liter of conjugate solution (see step 4.3) into all wells of micro plate .cover the plate with a new sealing tape and incubate 30 min at 37c .don't place the plates in pile.

5.5. Aspirate off the contents of wells with washer. Wash micro plate 5 times by the washing solution and dry as described in step 5.1

5.6. Dispense 100 micro liter of Chromogen solution into all wells of micro plate. Put the micro plate in dark place and incubate 10 min at room temperature. Don't place the plates in pile.

5.7. Stop the enzymatic reaction by adding 50 micro liter of stop solution to each well. Plates should be read immediately. Measure the absorbance at 450 nm.

6.CALCULATION AND INTERPRETATION OF RESULTS

The assay should be considered as correct when:

- The value of optical density (OD) in conjugate control well is not more than 0.15;
- The mean value of OD in Negative control serum wells for Ag HCV is not more than 0.20
- The mean value of OD in Positive Control serum wells for Ag HCV is not less than 1.5;

If any of these demands are not kept, the test should be repeated.

Calculate the mean values of negative control and the positive control, then calculate the cut-off value through the following formula:

$$\text{NC} + 0.20 = \text{CUT-OFF}$$

Samples with an OD within the cut – off $\pm 10\%$ are considered in a gray zone . Patients in this zone should be followed up.

Samples with OD values higher than the upper limit of the gray zone are to be considered positive (reactive with HCV antigens)

Samples with an OD values lower than the lower limit of the gray zone are to be considered negative (not reactive with HCV antigens)