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Human Hepatitis C virus IgM ELISA Kit

to determine Human Hepatitis C Virus antibody IgM in Serum, Blood Plasma, Saliva, Urine, CSF And Other Related Tissue Liquid Samples.

INSTRUCTION MANUAL

FOR ELISA KIT No: LTuH2851DEA

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INTENDED USE

This kit is used to assay the Hepatitis C virus IgM(HCV-IgM) in Human serum, blood plasma, and other related tissue Liquid.

MANUAL VERSION 1.04

PRINCIPLE

The kit uses a qualitative enzyme-linked immunosorbent assay (ELISA) to detect the presence of Human Hepatitis C virus IgM (HCV-IgM) in samples. Samples containing Human Hepatitis C virus IgM (HCV-IgM) are added to pre-coated microwell followed by incubation and washing. Antibodies in the samples bind to the antibody coated on the plate. Unbound antibody is washed away during the washing step. A Horseradish Peroxidase (HRP) conjugated detection antigen is then added, incubated and washed. Finally, Substrate Solution A and B are added which change color into blue, and to yellow in presence of an acid. By reading the absorbance of the solutions at 450nm wavelength and comparing it with the threshold value, the existence of the Human Hepatitis C virus IgM(HCV-IgM) in the sample can be determined.

MATERIALS SUPPLIED IN THIS KIT

1	Positive control	1 Vial	7	Substrate Solution A	1 Vial
2	Negative control	1 Vial	8	Substrate Solution B	1 Vial
3	Coated Plate	8X12strips	9	Stop Solution	1 Vial
4	HRP-Conjugated Reagent	1 Vial	10	Instruction Manual	1
5	20×wash solution	25ml X 2	11	Plate Seal	3
6	Sample diluent	1 Vial	12	Zipper Bag	1

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 ℃ incubator
2. Standard Enzyme reader
3. Precision pipettes and Disposable pipette tips
4. Distilled water
5. Disposable tubes for sample dilution
6. Absorbent paper

IMPORTANT NOTES

1. Before using, keep the kit outside and allow it to come to room temperature. After breaking the seal of ELISA coated-plate, keep the unused strips in the zipper bag at 2-8℃.
2. Pipette tips and seal plate membrane should not be used more than once in order to avoid cross contamination.
3. All samples and all discard generated should be disposed as per local rules.
4. Reagents of different batches must not be mixed and should be used before their respective validity dates.
5. Substrate B is sensitive to light and therefore should not be exposed to light for too long.
6. It is recommended that all standards, test samples are run at least in duplicates. If the testing material content in the sample is excessively high，then please use sample dilution solution to dilute the samples multiple-folds and multiply the Optical Density reading total dilution times accordingly at the time of calculation.

SAMPLE PREPARATION

1. Samples containing NaN_3 are not recommended for testing with ELISA as they may inhibit the activity of Horse Radish Peroxidase (HRP).

2. After extraction, experiment should be conducted immediately. Otherwise, keep the sample at -20℃. Avoid repeated freeze-thaw cycles.

3. Serum: Allow the sample to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RCF) for 20 minutes. Collect the supernatant carefully. In case of storage, centrifugation should be performed again prior to use.

4. Blood plasma: During sample collection EDTA or sodium citrate should be used for anti-coagulation. Centrifuge (at 2000-3000 RCF) for approximately 30 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.

5. Urine: Collect the sample in a sterile tube. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.

6.Cell culture supernatant: For secreted components, centrifuge (at 2000-3000 RCF) for approximately 20 minutes and collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells by repeated freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes and collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.

7.Tissue sample: Incise tissue sample of interest and add few mls of PBS (pH 7.4). Freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8°C. Add few mls of PBS (pH 7.4) and then homogenize the sample. Centrifuge (at 2000-3000 RCF) for 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use.

8.CSF: It is recommended that freshly collected Cerebrospinal Fluid should be proceeded for immunological examination immediately without any storage or waiting for other microscopic/bacterial/chemical examinations. Fluid to be centrifuged at 1000-2000 RCF for 20 minutes, and supernatant should be used for further examination. In case of storage, centrifuge again prior to use.

ASSAY PROCEDURE

1. Determine the number of strips required for the assay. Insert the strips in the Microplate frame for use. The unused strips should be stored at 4°C for up to one month. Label the sample wells, negative control wells, positive control wells and at least 1 blank well.
2. Add 100µl of Sample Diluent to each well except the Blank Well.
3. Add 10µl of Sample or 10µl of negative control or 10µl of positive control in respective wells.
4. Set the Blank wells without any solution and Incubate for 60 minutes at 37 ℃.
5. Dilute the washing concentrate (20X) with distilled water.
6. Remove the seal plate membrane carefully, drain the liquid in the wells and shake off the remaining liquid by tapping on an absorbent paper. Fill each well with washing solution. Drain the liquid after 60 seconds of soaking. Then repeat this procedure five times and blot the plate.
7. Add 100µl HRP-conjugate reagent to each well, except the blank well. Mix gently and incubate for 30 minutes at 37 ℃.
8. Drain the liquid in the wells and shake off the remaining liquid by tapping on an absorbent paper. Fill each well with washing solution. Drain the liquid after 60 seconds of soaking. Then repeat this procedure five times and blot the plate.

9. Add 50µl Substrate Solution A firstly to each well. Then add 50µl Substrate Solution B to each well. Shake gently to mix the wells and incubate for 10 minutes at 37°C away from light for color development. Shades of blue can be seen in the positive controls while the negative controls show no obvious colors. Users may increase this incubation time up to 30 minutes, if required.
10. Add 50µl Stop Solution to each well to stop the reaction (the blue color changes into yellow immediately at that moment).
11. Set blank well as zero absorbance and measure the absorbance (OD) of each well at 450nm wavelength using a microplate reader. This reading should be taken within 10 minutes after having added the stop solution.

RESULT DETERMINATION

Test validity: the mean of Positive control wells≥0.50 and the mean of Negative control wells < 0.50.

Calculate Threshold: Threshold = (the mean of Negative control wells) **X** 2.8

If the mean of Negative Controls is less than 0.05, then use the threshold as 0.14

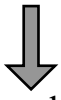
Test Sample Status Determination:

Sample Status Negative: If Test Sample OD< Threshold.

Sample Result Positive: If Test Sample OD≥ Threshold

PROTOCOL SUMMARY

Label microplate wells and prepare 1X washing buffer as required.



Add samples and controls (as detailed in protocol) in respective wells and incubate for 60 minutes at 37°C.

Wash the plate five times. Add HRP-Conjugate Reagent and incubate for 30 minutes at 37°C.

Wash the plate five times. Add Substrate Solution A and B. Incubate for 10-30 minutes at 37 °C for color development.

Add stop solution

Read the OD value within 10 minutes.

Calculate and determine result.

PACKAGE SIZE：96Tests.

VALIDITY & STORAGE: Six months (at 2-8°C) or Twelve months (at -20°C) from date of purchase. Once opened, kit to be consumed in the shortest period. Avoid repeated freeze thaw cycles.