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Human Hepatitis G Virus (HAG) ELISA Kit

Size: 96 T, 48T

Catalogue Number:QS3839Hu

Assay Time: 60 minutes

Store all reagents at 2-8℃/-20°C

Validity Period: 2-8℃ for six months, -20°C for one year. Avoid repeated thaw cycles.

For samples: In serum, plasma, culture media or any biological fluid.

FOR RESEARCH USE ONLY！

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS！

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING！

Human Hepatitis G Virus (HAG) ELISA Kit

FOR RESEARCH USE ONLY

Purpose

Our Human Hepatitis G Virus (HAG) ELISA Kit is to assay the qualitative determination of HAG in Human serum, plasma, culture media or any biological fluid.

Principle

The ELISA is based on the the qualitative enzyme immunoassay technique.The Microplate provided in this kit has been pre-coated with an antigen specific to HAG, make it to solid-phase antigen.Samples are added to the Microplate wells and combined to the specific antigen. Then a Horseradish Peroxidase (HRP)-conjugated antigen specific for HAG is added to each Microplate well and incubated,so the antigen-antibody-Enzyme labeled antigen complex is formed.Following a wash to remove any unbound reagent,then the TMB substrate solution is added to each well. Only those wells that contain HAG and HRP conjugated HAG antigen will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The qualitative determination of HAG is determined by comparing with the CUT OFF value.

Materials provided with the kit

	Materials provided with the kit	96 determinations	48 determinations
1	User manual	1	1
2	Closure plate membrane	2	2
3	Sealed bags	1	1
4	Microelisa stripplate	1	1
5	Negative control	0.5ml×1 bottle	0.5ml×1 bottle
6	Positive control	0.5ml×1 bottle	0.5ml×1 bottle
7	HRP-Conjugate reagent	6ml×1 bottle	3ml×1 bottle
8	Sample diluent	6ml×1 bottle	3ml×1 bottle
9	Chromogen Solution A	6ml×1 bottle	3ml×1 bottle
10	Chromogen Solution B	6ml×1 bottle	3ml×1 bottle

11	Stop Solution	6ml×1 bottle	3ml×1 bottle
12	wash solution	20ml (30X)×1bottle	20ml (20X)×1bottle

Sample preparation

1. **Serum preparation**

   After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again.

2. **Plasma preparation**

   Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifugated for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifugated again.

3. **Urine samples**

   Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.

4. **Cell samples**

   If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X100/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again.

5. **Tissue samples**

   Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80℃ for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be

operated at 4 ℃ . Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

Notes:

1. Sample extraction and ELISA assay should be performed as soon as possible after sample collection. The samples should be extracted according to the relevant literature. If ELISA assay can not be performed immediately, samples can be stored at -20 ℃ .Repeated freeze-thaw cycles should be avoided.
2. Our kits can not be used for samples with NaN3 which can inhibit the activity of HRP.

Procedure

1. In the Microplate, number the corresponding micropores of the sample in sequence,leave two wells as negative control, two wells as positive control and one empty well as blank control. (blank control hole dont add samples and HRP-Conjugate reagent, the rest step operation are same)
2. **Add sample:** In the Microelisa stripplate, leave a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. In the Microplate, number the corresponding micropores of the sample in sequence,leave two wells as negative control, two wells as positive control and one empty well as blank control. (blank control hole dont add samples and HRP-Conjugate reagent, the rest step operation are same)
4. **Add HRP:** Add 50µl HRP-Conjugate reagent to each well except the blank control well.
5. **Incubation:** incubate 30 min at 37℃ after sealed with Closure plate membrane.
6. **Preparing solution:** Dilute the concentrated Wash Solution with distilled water (30 times for 96T and 20 times for 48T).
7. **Washing:** Carefully peel off Closure plate membrane, aspirate and refill with Wash Solution (350µl to 400µl, or fill it completely, overflow is acceptable) .Remove the Wash Solution after resting for 30 seconds. Repeat the washing procedure for 5 times. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Invert the plate and blot it against clean

paper towels.

8. **Chromogenic reaction:** Add 50 µl Chromogen Solution A and 50µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 10min. Protect from light.
9. **Stop the reaction:** Add 50µl Stop Solution to each well to terminate the reaction. The color in the wells should change from blue to yellow.
10. **Assay:** Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15min after adding stop solution.

Notes:

1. Store the kit at upon receipt.The kit should be equilibrated to room temperature before the assay. Remove any unneeded strips from Human HAG antigen-Coated plate, reseal them in zip-lock foil and keep at 2-8℃/-20° C.
2. Precipitates may appear in concentrated washing buffer. Please heat the buffer to dissolve all the precipitates, which will not affect the results.
3. Accurate pipette should be used to avoid experimental error. Samples should be added to the Microplate in less than 5 minutes. If a large number of samples are included, multiple channel pipette is recommended.
4. Standard curve should be included in every assay. Replicate wells are recommended. If the OD value of the sample is greater than the first well of standards, please dilute the sample (n times) before test. When calculating the original HAG concentration, please multiply the total dilution factor (XnX5).
5. In order to avoid cross-contamination, Microplate sealers are for one-time use only.
6. Please keep Substrate away from light.
7. All the operation should be accordance with the manufacturer's instructions strictly. The results determined by the Microtiter Plate Reader.
8. All the samples, washing buffer and wastes should be treated as infectious agents.
9. Reagents from different lots should not be mixed.

Determine the result

Test effectiveness: the average value of positive control ≥1.00; The average value of negative control ≤0.10.

The critical value (CUT OFF) calculation: critical value = the average value of negative control + 0.15

Negative judgement: if the OD value<CUT OFF, the sample is Human HAG negative.

Positive judgement: if the OD value ≥CUT OFF, the sample is Human HAG positive.

Notes

1. Store the kit at 2-8 ℃ /-20 ℃ upon receipt.The kit should be equilibrated to room temperature before the assay. Remove any unneeded strips from HAG antigen-Coated plate, reseal them in zip-lock foil and keep at 2-8℃/-20℃.
2. Precipitates may appear in concentrated washing buffer. Please heat the buffer to dissolve all the precipitates, which will not affect the results.
3. In order to avoid cross-contamination, Closure plate membranes are for one-time use only.
4. Please keep Substrate away from light.
5. Please keep Substrate away from light.
6. All the operation should be accordance with the manufacturer's instructions strictly. The results determined by the Microelisa stripplate Reader.
7. All the samples, washing buffer and wastes should be treated as infectious agents.
8. Reagents from different lots should not be mixed.

Storage and validity

1．Storage: 2-8℃/-20° C.

2．Duration: 2-8℃ for six months, -20° C for one year. Avoid repeated thaw cycles.