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(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)

Human Hepatitis D Virus (HDV) Antigen ELISA Kit

Catalog No: E-HD-E121

96T/96T*2

Version Number:	V1.2
Replace version:	V1.1
Revision Date:	2024.03.14

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.vetassay-elab.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This ELISA kit uses Sandwich-ELISA as the method to detect the Hepatitis D Virus (HDV) antigen in human serum or plasma. The ELISA Microtiter plate provided in this kit has been pre-coated with Human-anti-HDV antibody. Add samples and lysis buffer to the ELISA Microtiter plate wells, then add HRP conjugated anti-HDV. The HDV antigen in samples will be combined with the Human-anti-HDV antibody, and the combined HDV antigen will then specifically combine with the HRP conjugated anti-HDV. Free components are washed away. The substrate reagent is added to initiate the color developing reaction. The presence of HDV antigen can be determined according to the absorbance value by using a microplate reader with 450 nm (630 nm) wavelength.

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Lysis Buffer	6 mL
HRP Conjugate	6 mL
20×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Positive Control	1 mL
Negative Control	1 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other materials required but not supplied

Micro-plate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)

High-precision transferpettor, EP tubes and disposable pipette tips

37°C Incubator or water bath

Deionized water

Absorbent paper

Notes

1. Please read the manual carefully before use, changes of operation may result in unreliable results.
2. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.
3. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
4. The ELISA Microtiter plate obtained from cold storage conditions should be adjusted to room temperature before use. The unused plate should be kept in a sealed bag with desiccant.
5. Concentrated washing liquid at low temperature condition is easy to crystallization, it should be adjusted to room temperature in order to dissolve completely before use.
6. The results shall depend on the readings of the micro-plate Reader.
7. **Each reagent is optimized for use in the E-HD-E121. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-HD-E121 with different lot numbers.**
8. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

Storage and expiry date

Store at 2-8°C. Avoid freeze.

Please store the opened plate at 2-8°C, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

Sample preparation

1. **Serum/plasma:** Fresh collected serum samples should be fully centrifuged, then take clear liquid for test. Suspended fibrous protein may cause a false positive. Samples can be stored at 2-8°C for one week and stored at -20°C for more than a week. Avoid freeze-thaw cycles. Freezing samples should be mixed fully before test.
2. Anticoagulant (such as EDTA, sodium citrate and heparin, etc.) in samples do not affect the results. Substances in serum such as bilirubin, hemoglobin, high fat or concentration of proteins sample may not affect the results. HCV, HEV, rheumatoid factors and other related diseases usually do not affect the results.
3. **Wash Buffer:** The 20×**Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, and then dilute it with deionized water at 1:19.

Assay procedure

Restore all reagents and samples to room temperature (25°C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8°C.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 1 well for blank control, 3 wells for negative control and 2 wells for positive control. **Samples need test in duplicate** (Blank well is not necessary for dual-wavelength detection).
2. **Add sample:**
 - (1) Add 100 µL of **Positive/Negative Control** respectively to **Positive/Negative Control** wells, keep the blank control well empty.
 - (2) Dilute the tested **Serum/plasma** with **Lysis Buffer** at 1:1 into sample well (add 50 µL of lysis buffer and add 50 µL of sample), mix fully.
3. **HRP conjugate:** add 50 µL of **HRP Conjugate** to each well except the blank control well.
4. **Incubate:** gently tap the plate to mix thoroughly, cover the ELISA Microtiter plate with sealer. Incubate for 50 min at 37°C in shading light.
5. **Wash:** remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with **Wash Buffer** and immerse for 30-60 sec each time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
6. **Add substrate:** add 50 µL of **Substrate Reagent A** and 50 µL of **Substrate Reagent B** to each well. Gently tap the plate to mix thoroughly. Cover with a new plate sealer. Incubate for 10 min at 37°C in shading light.
7. **Stop reaction:** add 50 µL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
8. **OD Measurement:** set the Microplate Reader wavelength at 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) to detect A value of each well. Blank well is not essential when using dual wavelength 450 nm/630 nm for detection. **Note: Read the results within 10 min.**

Reference value

Normally, blank well (just substrate agent and stop solution) absorbance: $A_{450} \leq 0.08$;
positive control (PC): $A_{450} > 0.30$ and average A value of negative control (NC): $A_{450} < 0.10$.

Interpretation of test results

Cut Off = 0.10 + average A value of negative control (NC) (when average A_{450} of NC < 0.05 , calculate at 0.05; while average A value of NC ≥ 0.05 , calculate at the actual value).

1. Positive result: average A value of sample \geq Cut Off.
2. Negative result: average A value of sample $<$ Cut Off.
3. Negative result indicates no HDV-Ag detected in samples, while positive result means the opposite.

Limitations of test method

1. This test is only used as the qualitative detection of HDV-Ag in serum and plasma of human.
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.