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

Human Herpes Simplex Virus Type Ⅱ (HSV Ⅱ) IgG ELISA Kit

Catalog No: E-HD-E104

96T/96T*2

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 adopts Indirect-ELISA method as its principle. The ELISA Microtiter plate provided in this kit has been pre-coated with purified HSV-Ⅱ antigen. When samples are added into the ELISA Microtiter plate wells, the HSV-Ⅱ antibody in the sample will combine with the pre-coated antigen to form antigen-antibody compound. Free components are washed away. HRP conjugated Mouse anti human IgG monoclonal antibody is added to each well and react with the compound to form antigen-antibody-HRP antibody ompound. Free components are washed away. The TMB substrate is added to initiate the color developing reaction. The presence of HSV-Ⅱ-IgG can be determined according to the OD value after colorimetric assay with the micro-plate reader.

Kit components

Item	Specification
ELISA Microtiter plate	96 wells
Positive Control	0.5 mL
Negative Control	0.5 mL
HRP Conjugated Working Solution	12 mL
Sample Diluent	12 mL
20×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Plate Sealer	3 pieces
Sealed Bag	1
Manual	1 copy

Experimental instrument

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

High-precision transferpettor, EP tubes and disposable pipette tips

37℃ Incubator or water bath

Deionized water

Absorbent paper

Loading slot for Wash Buffer

Sample preparation

1. Fresh collected serum specimens should be fully centrifugal, then take clear liquid for test. The suspended fibrous protein may cause a false positive result if not fully precipitated.
2. Anticoagulant (EDTA, sodium citrate and heparin) in samples do not affect the result of the experiment in general. Endogenous interference substances in serum such as blood lipids, cholerythrin, hemoglobin, rheumatoid factors positive samples, AFP positive samples and pregnant samples may not affect the results. Common positive samples of specific virus antibodies, such as HAV, HBV, HCV, EB, HSV, RV and related diseases, will not affect the results.
3. Do not use heat inactivated samples, heat inactivation will degrade antibodies.
4. Samples should be stored in 2~8℃. If samples not tested in a week, store them at -20℃ and avoid freeze-thaw cycles.

Assay procedure

Bring all reagents to room temperature for 30 min. Dilute the 20×Concentrated Wash Buffer for 20 times with distilled water.

1. **Add sample:**
   a) Take out Micro-plate and mark it, reserve 1 well for blank control (empty), 3 wells for negative control, 2 well for positive control (100 μL control serum for each well). (Blank well is not necessary for dual-wavelength detection)
   b) Dilute the tested serum with Sample Diluent at 1:10 (add 100 μL of Sample Diluent to the reaction well, and then add 10 μL of serum sample), mix fully.
   c) Gently tap the plate to ensure thorough mixing.
2. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37℃.
3. **Wash:** After incubation, 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.
4. **HRP conjugate:** Add 100 μL of HRP Conjugate working solution to each well except the blank control well.
5. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37℃.
6. **Wash:** After incubation, 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.
7. **Add substrate:** Add 50 μL of Substrate Reagent A and 50 μL of Substrate Reagent B to each well. Gently tap the plate to ensure thorough mixing. Cover with a new plate sealer. Incubate for 15 min at 37℃ in dark.
8. **Stop reaction:** Add 50 μL of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
9. **OD Measurement:** Set the Micro-plate 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 needed when using dual wavelength 450 nm/630 nm for detection.

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 microplate reader.
7. **Each reagent is optimized for use in the E-HD-E105. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-HD-E105 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 the kit at 2~8℃. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2 - 8 ℃.

Expiry date: expiration date is on the packing box.

Reference value

1. **Result analysis**
   (1) Use each test result independently. Determine the result according to the Cut Off value.
   (2) Calculate the Cut Off: Cut Off(C.0) = 0.10 + negative control (NC) average A value (when NC average A_{450} ＜ 0.05, calculate at 0.05; while NC average A_{450} ≥ 0.05, calculate at the actual value).
2. **Quality control**
   (1) Blank well (just chromogenic agent and Stop Solution) absorbance ≤ 0.08.
   (2) Positive control (PC) A_{450} ＞ 0.30.
   (3) Negative control (NC) A_{450} ＜ 0.08.
   The experimental result is valid if quality control is valid.
3. **Determination of results**
   (1) Positive result: Sample absorbance ≥ Cut Off.
   (2) Negative result: Sample absorbance ＜ Cut Off.

Interpretation of test results

1. Negative result indicates no HSV-Ⅱ-IgG antibody detected in samples, while positive result is just

the opposite.

2. The positive result of HSV-Ⅱ-IgG antibody is an important index of HSV-Ⅱ infection

Limitations of test method

1. All high sensitivity immune experiment system exists potential non-specificity. Therefore, unacceptable positive results may be caused by biological false positive of ELISA method.
2. Any positive result should be combined with clinical information to determine the final result.

Notes

9. 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.
10. 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.
11. The ELISA 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.
12. 20×Concentrated Wash Buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
13. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
14. The tested sample should be kept fresh.
15. The results shall depend on the readings of the Micro-plate Reader.
16. Do not use components from different batches of kit.

Storage and shelf life

Store unopened at 2 to 8℃. Do not freeze.

Please store the opened kit at 2~8℃, protect from light and moisture. The shelf life of the opened kit is

up to 1 months.

Expiry date: expiration date is on the box.