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

Total IgE Antibodies ELISA Kit

Catalog No: E-HD-E189

96T

Version Number: V1.0

Revision Date: 2024.10.18

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

The method used in this kit has been developed to detect the level of total IgE in human serum, was Enzyme-Linked Immunosorbent Assay (ELISA). The anti-human-IgE antibody is coated to the microplates. The patient's serum samples are pipetted into the microplates and incubated. During this time, the IgE antibodies react with anti-human-IgE antibody and bind to the surface of the microplates via the anti-human IgE antibody. Non-bound material is removed by washing. After this, an anti-human IgE antibody coupled with the HRP conjugate solution [conjugate anti-IgE-horseradish peroxidase (HRP)] is added and incubated. This binds to the IgE in the test fields from the first incubation. Non-bound detection antibodies are removed by washing. Next, TMB color reagent (TMB+H₂O₂) is added and incubated, a specific enzymatic color reaction of the HRP takes place which results in the formation of blue color of the TMB color reagent in the microplates. The enzymatic reaction is stopped with adding the stop reaction solution, and the color of the TMB color reagent turn to yellow. The color intensity is directly proportional to the IgE antibody content of the serum sample. Read optical density (OD) of the microplates at 450nm, then calculate the total IgE.

Kit components

| Item | Specifications |
|-----------------------------|---|
| ELISA Microtiter plate | 96 wells |
| Sample Diluent | 15 mL |
| HRP Conjugate | 12 mL |
| TMB Colour Reagent | 12 mL |
| Standard Liquid | 1 mL each (1, 5, 10, 25, 50, 200 IU/mL) |
| Stop Solution | 12 mL |
| 20×Concentrated Wash Buffer | 30 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

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

High-precision transferpette, EP tubes and disposable pipette tips

37°C Incubator or water bath

Deionized or distilled 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-E189. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-HD-E189 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. Fresh **Serum** samples are recommended. Samples must not be bacteria contaminated or haemolysed or lipidic or icteric, or contain suspended material (fibrin).
2. Samples should not be kept at room temperature (20-25°C) for more than 8 hours, should not be kept at 2-8 °C for more than 48 hours. For long term storage, serum samples can be frozen at -20 °C. To avoid unreliable results caused by repeated freezing and thawing, serum sample should be aliquot in small vials without preservative.
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.
4. **Dilute Sample:** Dilute serum samples 1:10 with sample dilution (e.g. dilute the content of 10μl serum with 90μL Sample dilution).

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. Dispense into the respective wells 100 µL of **Standard Liquid** (1-5-10-25-50-200 IU/mL) and **Dilute Sample**; mix gently apply a sealing plate membrane and incubate for 45 minutes at 37 °C.
2. Aspirate the liquid from the wells and perform 5 washing cycles with 250 µL of **Wash Buffer** for each well (at the end the wells are empty). After the last wash, dry the microplates on thick tissue paper.
3. Dispense 100 µL **HRP conjugate** solution into each well, and incubate for 45 minutes at 37 °C.
4. Aspirate the liquid from the wells and perform 5 washing cycles with 250 µL of **Wash Buffer** for each well (at the end the wells are empty). After the last wash, dry the microplates on thick tissue paper.
5. Dispense 100 µL **TMB colour reagent** into each well, and incubate for 15-20 minutes at room temperature (20-25 °C) (avoid exposure to bright light).
6. Dispense 100 µL **Stop Solution** into each well following the same sequence used for the **TMB colour reagent**, in order to maintain the same incubation time for all the wells.
7. Read optical density (OD) at 450 nm. If available, use the dual wavelength measurement mode with 630 nm reference wavelength which eliminates any influence caused by the microplate itself (finger prints, scratches, dust etc.). The reading must be performed within 30 minutes from the end of the test.

Result analysis

Draw the calibration curve by plotting the values of the calibrators (IU/mL) on the abscissa axis (x) and the OD values on the ordinate axis (y). Thus, the concentration of diluted samples can be calculated by the OD value with the calibration curve. The final concentration of samples was the concentration of diluted samples multiply by 10 (the dilution factor).

Quality control limits:

- ① Absorbance of negative control (OD value) < 0.10;
- ② Absorbance of positive control (OD value) \geq 1.00.

Negative and positive judgments:

Positive : OD value \geq 87.0 IU/mL

Negative.: OD value < 87.0 IU/mL

Limitations of test method

1. In order to obtain the best precision for immunoassay, the technician should be well trained periodically and the pipettor should be well maintained and calibrated regularly.
2. Always use the same lot number of the reagents from the same lot number of assay kit.
3. There are some interference when serum sample is bacteria contaminated or haemolysed or lipemic or icteric.