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

Allergen-specific IgE Antibodies ELISA Kit (B)

Catalog No: GEH094

96T

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.

Tel: 400-967-3365
Email: techsupport@uni-science.com
Website: www.elabscience.cn

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 levels of allergen-specific IgE antibodies in human serum, was Enzyme-Linked Immunosorbent Assay (ELISA). The antigens are coated to the microplates. The patient's serum samples are pipetted into the microplates and incubated. During this time, the allergen-specific 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 detector antibodies are removed by washing. Next, TMB color reagent (TMB+H_2O_2) 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 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 OD at 450nm. OD values of samples are plotted on the calibration curve.

Test Items： House dust, cockroach, cat dander, dog dander, ragweed, mulberry tree, molds mixes (Penicilliurn chrysogenum, Alternaria alternate, Aspergillus fumigatus, Cladosporium herbarum), tree pollens mixes (oak, elm, sycamore, willow, cottonwood)

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Sample Diluent	5 mL
HRP Conjugate A:	0.6 mL
HRP Conjugate B	5.4 mL
TMB Colour Reagent	12 mL
Standard Liquid	1 mL
Negative Control	0.6 mL
Positive Control	0.6 mL
Stop Solution	12 mL
20×Concentrated Wash Buffer	30 mL
Calibrator	0.6 mL(100 IU/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 transferpettor, EP tubes and disposable pipette tips

37℃ Incubator or water bath

Deionized or distilled water

Absorbent pape

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 GEH094. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other GEH094 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℃. Avoid freeze.

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 (18-25℃） for more than 8 hours, should not be kept at 2-8℃ for more than 48 hours. For long term storage, serum samples can be frozen at -20℃. To avoid unreliable results caused by repeated freezing and thawing, serum sample should be aliquot in small vials without preservative.

Assay procedure

Warm up all reagents and serum samples to room temperature (18-25℃) for 30 minutes before use. Repack all unused microplates in the original hermetic bag and store at 2-8℃.

1. Reagent preparation:

A) Preparation of the washing solution:

Dilute the concentrated solution 1:20 with distilled or deionized H2O (e.g. dilute the content of one 30ml bottle with 570ml of H2O and mix gently).

B) Preparation of the HRP conjugate solution:

Dilute HRP conjugate A 1:10 with HRP conjugate B (e.g. dilute the content of 1ml HRP conjugate A with 9ml of HRP conjugate B and mix gently).

C) Preparation of the calibrator solutions:

Take 6 clean 1.5ml tubes marked 1, 2, 3, 4 and 5. Dilute the human IgE calibrator (100IU/ml) with sample dilution according to the following instruction:

Tube No.	Calibrator Solution added	Sample Dilution Volume added	Total Volume	Final Human IgE Concentration
1	100.00 IU/mL (0.25 mL)	0.25 mL	0.50 mL	50.00 IU/mL
2	50.0 IU/mL (0.20 mL)	0.37 mL	0.57 mL	17.50 IU/mL
3	100.00 IU/mL (0.25 mL)	0.80 mL	1.00 mL	3.50 IU/mL
4	100.00 IU/mL (0.25 mL)	0.80 mL	1.00 mL	0.70 IU/mL
5	50.0 IU/mL (0.20 mL)	0.20 mL	0.40 mL	0.35 IU/mL

(NOTE: This procedure can be omitted for qualitative assay.)

2. Testing procedure:

(1) After all reagents are at room temperature (18-25℃), secure the microplates onto the strip holding racks.

(2) Dispense 50μL sample into the respective wells for specific allergens, 50μL positive control, 50μL Negative control and 50uL calibrator solutions (0.35-0.70-3.50-17.50-50.00-100.00IU/mL) into corresponding wells; mix gently and incubate for 45 minutes at 37℃.

(3) Aspirate the liquid from the wells and perform 5 washing cycles with 250μL of washing solution for each well (at the end the wells are empty). After the last wash, dry the microplates on thick tissue paper.

(4) Dispense 50μL HRP conjugate solution into each well, and incubate for 45 minutes at 37℃.

(5) Aspirate the liquid from the wells and perform 5 washing cycles with 250μL of washing solution for each well (at the end the wells are empty). After the last wash, dry the microplates on thick tissue paper.

(6) Dispense 100μL TMB color reagent into each well, and incubate for 15-20 minutes at room temperature (avoid exposure to bright light).

(7) Dispense 100μL stop solution into each well following the same sequence used for the TMB color reagent, in order to maintain the same incubation time for all the wells.
(8) Read optical density (OD) at 450nm. If available, use the dual wavelength measurement mode with 620 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

Average cut-off OD value = 0.15+ negative control OD.
For quantitative assay, 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 samples' concentration can be calculated by the OD value with the calibration curve.

1. Quality control limits：

Absorbance of negative control (OD value) <0.10

Absorbance of positive control (OD value) ≥1.00

2. Negative and positive judgments：

Negative: OD less than 0.15

Positive: OD more than 0.15

3. Semi-quantitative level can be interpreted by comparing the reference standard values.

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. It is easy to get false positive result if washing procedure is not completed. As with all diagnostic tests, a definitive clinical diagnosis should not be made according to result of a single test. Diagnosis should only be made by the qualified physician after all clinical and laboratory findings have been evaluated.
3. Always use the same lot number of the reagents from the same lot number of assay kit.
4. There are some interference when serum sample is bacteria contaminated or haemolysed or lipidic or icteric.