IgE (Human) ELISA Kit

Catalog Number KA0216
96 assays
Version: 03

Intended for research use only
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Introduction

Intended Use

The Abnova IgE ELISA is intended for the quantitative determination of immunoglobulin E (IgE) concentration in human serum.

Background

Patients with atopic allergic diseases such as atopic asthma, atopic dermatitis, and hay fever have been shown to exhibit increased total Immunoglobulin E (IgE) levels in blood. IgE is also known as the reaginic antibody. In general, elevated levels of IgE indicate an increased probability of an IgE-mediated hypersensitivity, responsible for allergic reactions. Parasitic infestations such as hookworm, and certain clinical disorders including aspergillosis, have also been demonstrated to cause high levels of IgE. Decreased levels of IgE are found in cases of hypogammaglobulinemia, autoimmune diseases, ulcerative colitis, hepatitis, cancer, and malaria. Cord blood or serum IgE levels may have prognostic value in assessing the risk of future allergic conditions in children.

The IgE serum concentration in a patient is dependent on both the extent of the allergic reaction and the number of different allergens to which the patient is sensitized. Nonallergic normal individuals have IgE concentrations that vary widely and increase steadily during childhood, reaching their highest levels at age 15 to 20, and thereafter remaining constant until about age 60 when they slowly decline.

Principle of the Assay

The Abnova IgE Quantitative Test is based on a solid phase enzyme-linked immunosorbent assay (ELISA) The assay system utilizes one monoclonal anti-IgE antibody for solid phase (micorotiter wells) immobilization and goat anti-IgE antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the IgE antibody coated microtiter wells and incubated with the Zero Buffer at room temperature for 30 minutes. If human IgE is present in the specimen, it will combine with the antibodies on the well. The well is then washed to remove any residual test specimen, and IgE antibody labeled with horseradish peroxidase (conjugate) are added. The conjugate will bind immunologically to the IgE on the well, resulting in the IgE molecules being sandwiched between the solid phase and the enzyme-linked antibodies. After incubation at room temperature for 30 minutes, the wells are washed with water to remove unbound- labeled antibody. A solution of TMB Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of IgE is directly proportional to the color intensity of the test sample.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal anti-IgE coated microtiter plate with 96 wells.</td>
<td>1</td>
</tr>
<tr>
<td>Zero Buffer</td>
<td>13 ml</td>
</tr>
<tr>
<td>Enzyme Conjugate Reagent</td>
<td>18 ml</td>
</tr>
<tr>
<td>IgE reference standards, containing 0, 10, 50, 100, 400, and 800 IU/mL (WHO, 2nd IRP, 75/502). Liquid, 0.5 ml each</td>
<td>1 set</td>
</tr>
<tr>
<td>TMB Reagent (One-Step)</td>
<td>11 ml</td>
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<tr>
<td>Stop Solution (1N HCl)</td>
<td>11 ml</td>
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</table>

Storage Instruction

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above

Materials Required but Not Supplied

✅ Precision pipettes and tips, 20 µl, 100 µl and 150 µl.
✅ Distilled water.
✅ Disposable pipette tips.
✅ Vortex mixer, or equivalent.
✅ Absorbent paper or paper towel.
✅ A microtiter plat reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater.
✅ Graph paper.

Precautions for Use

- Limitation of procedure
- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
✓ Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
✓ The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
Assay Protocol

Reagent Preparation

All reagents should be allowed to reach room temperature (18-25°C) before use.

Sample Preparation

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 20µl of standards, samples, and controls into appropriate wells.
3. Dispense 100 µl of Zero Buffer into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing in this setup.
5. Incubate at room temperature (18-25°C) for 30 minutes.
6. Remove the incubation mixture by flicking plate content into a waste container.
7. Rinse and flick the microtiter plate 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 150µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature for 30 minutes.
11. Remove the incubation mixture by flicking well contents into sink.
12. Rinse the wells 5 times with running distilled or deionized water. (Please do not use tap water.)
13. Strike the wells sharply on absorbent paper to remove residual water droplets.
14. Dispense 100 µl TMB Substrate Reagent into each well. Gently mix for 10 seconds.
15. Incubate at room temperature, in the dark, for 20 minutes.
16. Stop the reaction by adding 100µl of Stop Solution to each well.
17. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
18. Read OD at 450nm with a microtiter well reader within 15 minutes.
Data Analysis

Calculation of Results

1. Calculate the average absorbance value (A450) for each set of reference standards, controls and samples.

2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in IU/mL on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal or X-axis.

3. Using the mean absorbance value for each sample, determine the corresponding concentration of IgE in IU/mL from the standard curve.

Example of standard curve
Results of a typical standard run with optical density readings at 450 nm shown in the y-axis against IgE concentrations shown in the x-axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own standard curve and patient data in each experiment.

<table>
<thead>
<tr>
<th>IgE (IU/mL)</th>
<th>Absorbance (450nm)</th>
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<tbody>
<tr>
<td>0</td>
<td>0.058</td>
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<tr>
<td>10</td>
<td>0.167</td>
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<td>50</td>
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<td>100</td>
<td>0.950</td>
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<tr>
<td>400</td>
<td>2.135</td>
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<td>800</td>
<td>2.748</td>
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Performance Characteristics

- Expected values

The total IgE level in a normal, allergy-free adults is less than 100 IU/ml in the serum. The minimum detectable concentration of IgE by the assay is estimated to be 5.0 IU/ml.
Resources

References

Plate Layout

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