ab108650

Immunoglobulin E (IgE) Human ELISA Kit

Instructions for Use

For the quantitative measurement of Immunoglobulin E (IgE) concentrations in Human serum

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

ab108650 Immunoglobulin E (IgE) Human ELISA Kit is intended for the quantitative determination of Immunoglobulin E (IgE) concentration in Human serum.

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 reagenic 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 he 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.
2. Assay Summary

*ab108650 is based on the principle of a solid phase enzyme-linked immunosorbent assay.*

The assay system utilizes one monoclonal anti-IgE antibody for solid phase (microtiter wells) immobilization and goat anti-IgE antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution.

The test sample is added to the IgE antibody coated microtiter wells and incubated with the Buffer at room temperature for 30 minutes. If Human IgE is present in the specimen, it will combine with the antibody 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 enzyme-linked antibodies. After incubation at room temperature for 30 minutes, the wells are washed with water to remove unbound labeled antibodies.

A solution of tetramethylbenzidine (TMB) reagent is added and incubated 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.
3. Kit Contents

- Monoclonal anti-IgE coated microtiter plate with 96 wells.
- Buffer, 13 ml.
- Enzyme Conjugate Reagent, 18 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.
- IgE Reference Standard 1 (0.5ml, 0 IU/ml)
- IgE Reference Standard 2 (0.5ml, 10 IU/ml)
- IgE Reference Standard 3 (0.5ml, 50 IU/ml)
- IgE Reference Standard 4 (0.5ml, 100 IU/ml)
- IgE Reference Standard 5 (0.5ml, 400 IU/ml)
- IgE Reference Standard 6 (0.5ml, 800 IU/ml)

4. Storage and Handling

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.
5. Additional Materials Required

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

6. Preparation of Reagents

All reagents should be allowed to reach room temperature (18-25°C) before use.
7. Preparation and Collection of Specimen

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.

8. Assay Method

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 20 μl of standards, specimens, and controls into appropriate wells.
3. Dispense 100 μl of Buffer into each well.
4. Thoroughly mix for 30 seconds. It is very important to have a 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 150 μl of Enzyme Conjugate Reagent into each well.
10. Gently mix for 10 seconds
11. Incubate at room temperature for 30 minutes.

12. Remove the incubation mixture by flicking plate contents into sink.

13. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)

14. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.

15. Dispense 100 μl TMB Reagent into each well. Gently mix for 10 seconds.

16. Incubate at room temperature in the dark for 20 minutes.

17. Stop the reaction by adding 100 μl of Stop Solution to each well.

18. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

19. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.
9. Data Analysis

1. Calculate the average absorbance value \( (A_{450}) \) 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 linear graph paper, with absorbance on the vertical \((y)\) axis and concentration on the horizontal \((x)\) axis.

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

A. Typical Data

Results of a typical standard run with absorbency readings at 450nm shown on the Y axis against IgE concentrations shown on the X axis.

**NOTE:** This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.
<table>
<thead>
<tr>
<th>IgE (IU/ml)</th>
<th>Absorbance (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.058</td>
</tr>
<tr>
<td>10</td>
<td>0.167</td>
</tr>
<tr>
<td>50</td>
<td>0.538</td>
</tr>
<tr>
<td>100</td>
<td>0.950</td>
</tr>
<tr>
<td>400</td>
<td>2.135</td>
</tr>
<tr>
<td>800</td>
<td>2.748</td>
</tr>
</tbody>
</table>
B. Sensitivity

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

10. Limitations

- 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.
- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
# Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
</tr>
<tr>
<td></td>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td>----------</td>
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<td>-------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
</tr>
<tr>
<td></td>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
</tr>
<tr>
<td></td>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
</tr>
<tr>
<td></td>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
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For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).
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