ab108650
Immunoglobulin E (Inge) Human ELISA Kit

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

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

ab108650 Immunoglobulin E (Inge) Human ELISA Kit is intended for the quantitative determination of Immunoglobulin E (Inge) 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 (Inge) levels in blood. Inge is also known as the reagenic antibody. In general, elevated levels of Inge indicate an increased probability of an Inge-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 Inge. Decreased levels of Inge are found in cases of hypogammaglobulinemia, autoimmune diseases, ulcerative colitis, hepatitis, cancer, and malaria. Cord blood or serum Inge levels may have prognostic value in assessing the risk of future allergic conditions in children. The Inge 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 Inge 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. Protocol Summary

Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed

\[
\text{Add 100 } \mu\text{l of Buffer into each well. Mix thoroughly.}
\]

\[
\text{Incubate at room temperature.}
\]

\[
\text{Aspirate and wash each well. Add Enzyme Conjugate Reagent into each well and incubate.}
\]

\[
\text{Aspirate and wash each well. Add TMB Reagent into each well and incubate in the dark.}
\]

\[
\text{Add stop solution to each well, gently mix, and read the optical density at 450nm.}
\]
3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.
5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
- 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.
## 6. Materials Supplied

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal anti-Inge coated microtiter plate with 96 wells.</td>
<td>1</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Buffer</td>
<td>13 ml</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Enzyme Conjugate Reagent</td>
<td>18 ml</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>TMB Reagent (One-Step),</td>
<td>11 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Stop Solution (1N HCl)</td>
<td>11 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Inge Reference Standard 1</td>
<td>(0.5ml, 0 IU/ml)</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Inge Reference Standard 2</td>
<td>(0.5ml, 10 IU/ml)</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Inge Reference Standard 3</td>
<td>(0.5ml, 50 IU/ml)</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Inge Reference Standard 4</td>
<td>(0.5ml, 100 IU/ml)</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Inge Reference Standard 5</td>
<td>(0.5ml, 400 IU/ml)</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Inge Reference Standard 6</td>
<td>(0.5ml, 800 IU/ml)</td>
<td>+2-8°C</td>
</tr>
</tbody>
</table>
7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 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
8. Technical Hints

- This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.

- Make sure all buffers and solutions are at room temperature before starting the experiment.

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.

- Avoid foaming or bubbles when mixing or reconstituting components.

- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.

- Ensure plates are properly sealed or covered during incubation steps.

- Make sure you have the right type of plate for your detection method of choice.

- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.

- All samples should be mixed thoroughly and gently.

- Avoid multiple freeze/thaw of samples.

- Incubate ELISA plates on a plate shaker during all incubation steps.

- To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.
9. Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 All Reagents are ready to use as supplied.

9.2 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.
10. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.

- For each assay performed, a minimum of two wells must be used as the zero control.

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

- Differences in well absorbance or "edge effects" have not been observed with this assay.

11. Assay Procedure

11.1 Secure the desired number of coated wells in the holder.

11.2 Dispense 20 μl of standards, specimens, and controls into appropriate wells.

11.3 Dispense 100 μl of Buffer into each well.

11.4 Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.

11.5 Incubate at room temperature (18-25°C) for 30 minutes.

11.6 Remove the incubation mixture by flicking plate content into a waste container.

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

11.8 Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.

11.9 Dispense 150 μl of Enzyme Conjugate Reagent into each well.
11.10 Gently mix for 10 seconds.

11.11 Incubate at room temperature for 30 minutes.

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

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

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

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

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

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

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

11.19 Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.
12. Calculations

12.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

12.2 Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

   \[ \Delta \text{ Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.} \]

12.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

12.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.
13. Typical Data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

<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>

Figure 1: Results of a typical standard run with absorbency readings at 450nm shown on the Y axis against Inge concentrations shown on the X axis.
14. Assay Specificity

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

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Precision</td>
<td>Use of expired components</td>
<td>Check the expiration date listed before use. Do not interchange components from different lots</td>
</tr>
<tr>
<td></td>
<td>Splashing of reagents while loading wells</td>
<td>Pipette properly in a controlled and careful manner</td>
</tr>
<tr>
<td></td>
<td>Inconsistent volumes loaded into wells</td>
<td>Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance</td>
</tr>
<tr>
<td></td>
<td>Insufficient mixing of reagent dilutions</td>
<td>Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions</td>
</tr>
<tr>
<td></td>
<td>Improperly sealed microplate</td>
<td>Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of the ELISA kit</td>
<td>Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Poor standard curve</strong></td>
<td>Inaccurate Pipetting</td>
<td>Check pipettes</td>
</tr>
<tr>
<td></td>
<td>Improper standard dilution</td>
<td>Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing</td>
</tr>
<tr>
<td><strong>Low Signal</strong></td>
<td>Incubation times too brief</td>
<td>Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation</td>
</tr>
<tr>
<td></td>
<td>Inadequate reagent volumes or improper dilution</td>
<td>Check pipettes and ensure correct preparation</td>
</tr>
<tr>
<td></td>
<td>Incubation times with TMB too brief</td>
<td>Ensure sufficient incubation time until blue color develops prior addition of Stop solution</td>
</tr>
<tr>
<td><strong>Large CV</strong></td>
<td>Plate is insufficiently washed</td>
<td>Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Prepare fresh wash buffer</td>
</tr>
</tbody>
</table>
16. Notes
Technical Support

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