**Product Information**

**Immunoglobulin E (IgE) ELISA**

Catalog Number SE120068  
Storage Temperature 2–8 °C

**TECHNICAL BULLETIN**

**Product Description**

IgE constitutes a fraction of the total antibodies found in serum, 50-300 ng/mL compared to 10 mg/mL for IgG, and together with its Fc receptor is important in primary immune responses. The immunogenetic mechanisms underlying IgE responsiveness seen in the atopic diseases can be divided into antigen-specific and non-antigen-specific responses. IgE antibodies to common antigens are reported in the serum of 13% of normal blood donors. Autoantibodies to the IgE Fc-epsilon-RII (high affinity receptors) reported in the sera of patients with chronic urticaria, can induce histamine release from mast cells. 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. Certain groups of white blood cells, including basophils and tissue mast cells, have membrane receptors for the IgE molecule.

The Immunoglobulin E (IgE) ELISA Kit is intended for the quantitative measurement of IgE in human serum. The Immunoglobulin E (IgE) ELISA is a two-site sandwich ELISA method. Samples and diluent are added to the wells coated with Anti-IgE MAb. IgE in the serum binds to anti-MAb on the well. Unbound proteins are washed off by wash buffer. Anti-IgE HRP labeled second antibody is then adds. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of IgE in the samples. A standard curve is prepared relating color intensity to the concentration of the IgE.

**Components**

<table>
<thead>
<tr>
<th>Materials Provided</th>
<th>96 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwell coated with IgE MAb</td>
<td>12 x 8 x 1</td>
</tr>
<tr>
<td>IgE Standard: 6 vials (ready to use)</td>
<td>0.5 mL</td>
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<tr>
<td>IgE Enzyme Conjugate: 1 bottle (ready to use)</td>
<td>12 mL</td>
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<tr>
<td>Assay Diluent: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>TMB Substrate: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>Stop Solution: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>20x Wash concentrate: 1 bottle</td>
<td>25 mL</td>
</tr>
</tbody>
</table>

**Reagents and Equipment Required but Not Provided.**

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbent paper or paper towel
- Graph paper

**Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.
Preparation Instructions

Sample Preparation
1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2–8 °C) for 5 days. If storage time exceeds 5 days, store frozen at (−20 °C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

20x Wash Buffer Concentrate
Prepare 1x Wash buffer by adding the contents of the bottle (25 mL, 20x) to 475 mL of distilled or deionized water. Store at room temperature (18–26 °C).

Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that serum samples be run in duplicate.

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Pipette 20 µL of IgE standards, controls, and sera.
3. Add 100 µL of assay diluent into each well.
4. Cover the plate and incubate for 30 minutes at room temperature (18–26 °C).
5. Remove liquid from all wells. Wash wells three times with 300 µL of 1x wash buffer. Blot on absorbent paper towels.
6. Add 100 µL of Enzyme Conjugate into each well.
7. Cover the plate and incubate for 30 minutes at room temperature (18–26 °C).
8. Remove liquid from all wells. Wash wells three times with 300 µL of 1x wash buffer. Blot on absorbent paper towels.
9. Add 100 µL of TMB substrate to all wells.
10. Incubate for 10 minutes at room temperature.
11. Add 50 µL of stop solution into each well. Shake the plate gently for 30 seconds to mix the solution. Make sure that the blue color completely changes to yellow.
12. Read absorbance on ELISA Reader at 450 nm within 15 minutes from adding stop solution.
Results

Calculations

The standard curve is constructed as follows:

1. Check IgE standard value on each standard vial. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. To construct the standard curve, plot the absorbance for the IgE standards (vertical axis) against its concentration in IU/mL (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Use the absorbance for controls and each unknown sample to determine the corresponding concentration of IgE from the standard curve.

Expected Values

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for IgE may be used as initial guideline ranges only:

IgE Normal Range:
   Male <250 IU/mL,
   Female <175 IU/mL

Note: The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient’s history, physical findings and other diagnostic procedures.

References