An immunoblotting assay for the quantitative determination of Allergen Specific Immunoglobulin E (IgE) in human serum. For professional in vitro diagnostic use only.

**INTENDED USE**

The Allergen Test Kit is an immunoblotting assay for the qualitative determination of Allergen Specific Immunoglobulin E (IgE) in human serum.

**SUMMARY**

A national survey in the United States found that 56.4% of all U.S. citizens test positive to one or more allergens. A steadily increase in the prevalence of allergic diseases globally has occurred with about 30-40% of the world population now being affected by one or more allergic conditions. Immunoglobulin E (IgE) is specifically important to food allergies that is part of Type I hypersensitivity. Ordinarily IgE is produced to fight infection caused by parasites. This molecule is also produced in harmless things such as pollen, dust and foods that may cause allergic diseases like asthma, allergic rhinitis and food allergy.

**PRINCIPLE**

The Allergen Test Kit is an immunoblotting assay for the qualitative detection of IgE antibody to Allergen in human serum. The surface of nitrocellulose membranes of test strips are coated with specific allergens. During testing, the specimen is added to the test strip and then incubated. If the specimen contains allergen-specific IgE antibodies, it can react with the allergens and bind to the nitrocellulose membrane of the strip. Unbound material is removed by washing. Detector antibodies (Anti-human IgE antibody coupled with Biotin) are then added to the strip and incubated. The Detector antibodies will bind to the respective specific IgE which are bound to the strip in the first incubation. Unbounded Detector antibodies are removed by washing. Next, Streptavidin conjugated with Alkaline Phosphatase is added to the strip and incubated. This will bind to the Biotin which are bound to the strip in the second incubation. Unbound Streptavidin conjugate is removed by washing. Then substrate is added and incubated, which will cause a specific enzymatic color reaction of the Alkaline Phosphatase.

**WARNINGS AND PRECAUTIONS**

- For professional in vitro diagnostic use only. Do not use after expiration date.
- Do not mix reagents from different kits.
- Avoid cross contamination between reagents to ensure valid test results.
- Human specimens should be considered potentially hazardous and handled using established good laboratory working practices.
- Some components of this kit contain ProClin™ 300. Avoid any contact with skin or eyes.
- Conjugate contains Methylisothiazolone and Bromonitridodxane in sub toxic concentrations as preservatives.
- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection while handling reagents and specimens. Wash hands thoroughly when finished.
- Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not mouth pipette.
- Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
- Neutralized and acids or other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to a 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

**STORAGE AND STABILITY**

- Unopened test kits should be stored at 2-8°C upon receipt. All unopened reagents are stable through the expiration date printed on the box if stored between 2-8°C. Once opened, all reagents are stable for up to 1 month after the first opening date if stored between 2-8°C. Return reagents to 2-8°C immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution to a water bath at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- Do not expose reagents, especially the Substrate, to strong light or hypochlorite fumes during storage or incubation steps. If the substrate becomes colored, it will no longer be suitable for use.

**SPECIMEN COLLECTION PREPARATION**

- This kit can be performed using only human serum specimens. The blood samples should be acquired using venipuncture and separating the serum out after coagulation (30-40 minutes) by centrifugation for 10 minutes at 4000g.
- Separate serum from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipemic or turbid samples should not be used. Specimens with extensive particulates should be clarified by centrifugation prior to use. Do not use specimens with fibrin particles or contaminated with microbial growth.
- Do not leave specimens at room temperature for prolonged periods. Specimen should be stored at 2-8°C prior to assaying. For long term storage, specimens should be kept frozen at temperatures below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

**REAGENTS AND COMPONENTS**

<table>
<thead>
<tr>
<th>No.</th>
<th>Reagent</th>
<th>Component Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Test Strips</td>
<td>Test strips in plastic reaction troughs, nitrocellulose membranes coated with allergen material</td>
<td>10 x 20 mL</td>
</tr>
<tr>
<td>2</td>
<td>Detector Antibody</td>
<td>Bottle with white cap, ready for use.</td>
<td>1 x 4 mL</td>
</tr>
<tr>
<td>3</td>
<td>Conjugate</td>
<td>Bottle with red cap, ready for use.</td>
<td>1 x 4 mL</td>
</tr>
<tr>
<td>4</td>
<td>Substrate</td>
<td>Bottle with black cap, ready for use.</td>
<td>1 x 4 mL</td>
</tr>
</tbody>
</table>

**DIRECTIONS FOR USE**

- Freshly distilled or deionized water
- Graduated cylinders for wash buffer dilution
- 500 mL laboratory wash bottle
- Shaker
- Incubation box (for incubation in the dark)
- Vortex mixer
- Timer
- Hairdryer
- Disposable gloves
- Disposable reagent reservoirs

**NOTE:** Use only the components with the same lot number.

<table>
<thead>
<tr>
<th>Location</th>
<th>Abbreviation</th>
<th>Test Items</th>
<th>Test Items</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>To check if the test has been carried out properly.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>D1/D2</td>
<td>Der pteronyssinus, Der farinae.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>W1/W6</td>
<td>Short ragweed, Mugwort.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>E1/E5</td>
<td>Cat dander, Dog dander.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Spacing</td>
<td>(Light red color)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>B</td>
<td>Cockroach</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M1/M2/M3/M6</td>
<td>Penicillium Chrysogenum, Gladosporium Herbarum, Aspergillus Fumigatus, Alternaria Alternata</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>W</td>
<td>Japanese Hog</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>F1</td>
<td>White Oak, Elm, Sycamore, Willow, Cottonwood</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Spacing</td>
<td>(Dark red color)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>F1</td>
<td>Egg white</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>F2</td>
<td>Cow's milk</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>F3/F24/F23</td>
<td>Fish, Shrimp, Crab</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>F27/F88</td>
<td>Beef, Mutton</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>F20/27/F34/F37</td>
<td>Cashew nut, Peanut, Soybean</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>F11</td>
<td>Mango</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>F12</td>
<td>Wheat</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>F13</td>
<td>Total IgE</td>
<td></td>
</tr>
</tbody>
</table>

**Materials Required But Not Provided**

- Disposable tubing
- Hairdryer
- Incubation box (for incubation in the dark)
- Vortex mixer
- Timer
- Hairdryer
- Disposable gloves
- Disposable reagent reservoirs
VALIDATION REQUIREMENTS AND QUALITY CONTROL

The substrate incubation must be carried out in the dark to avoid auto-coloration of the substrate. It is strongly recommended to incubate the Test Strips in an incubation box during each incubation process.

Step Detailed Procedure
1. Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Dilute 1 volume of Concentrated Wash Buffer (25x) with 24 volumes of freshly distilled or deionized water in a wash bottle. Mix well before use.
2. Add 250 µL of the Working Wash Buffer into the reaction trough; wet the reaction trough by shaking it (on a shaker) at room temperature (18–25°C) for 5 minutes. Remove the Working Wash Buffer and wipe the excess water on the plastic surface using absorbent tissue.
3. First Incubation: Add 250 µL of serum specimens into the reaction troughs then incubate on the shaker at room temperature (18–25°C) for 45 minutes. Remove the serum specimens after incubation.
   NOTE: Discard the serum specimen carefully and avoid cross contamination of the incubation trough.
4. Wash test strips 6 times with Working Wash Buffer while holding the reaction trough diagonally. Shake the solution in the trough manually for about 10 seconds and then remove the Working Wash Buffer. Wipe the excess solution on the plastic surface using absorbent tissue.
   NOTE: Complete washing is important. Shake the Working Wash Buffer in the trough for several seconds before pouring it out to ensure effective washing.
5. Second Incubation: Add 5 drops (250 µL) of the Detector Antibody then incubate on the shaker at room temperature (18–25°C) for 45 minutes. Remove excess Detector Antibody after incubation.
7. Third Incubation: Add 5 drops (250 µL) of Conjugate then incubate on the shaker at room temperature (18–25°C) for 20 minutes. Remove excess Conjugate after incubation.
9. Fourth Incubation: Add 5 drops (250 µL) of Substrate then incubate (in the dark) on the shaker at room temperature (18–25°C) for 20 minutes in the dark. Remove excess Substrate after incubation.
   NOTE: The substrate incubation must be carried out in the dark to avoid auto-coloration of the substrate.
10. Rinse the strip under flowing water to stop the substrate reaction. Wipe the excess water on the plastic surface using absorbent tissue.
11. Dry the strip in the air or by using a conventional hair dryer which will speed up the drying process. The blue-purple color of the background disappears as the test strip dries.
   NOTE: The Test Strip should be dried completely before reading results.
12. Compare the strip with the color chart. A qualitative evaluation of those lines is possible by visual comparison of the color intensity of each line on the strip with the color chart.
   NOTE: The control line should appear now, which is used to check if the test has been carried out properly.

INTERPRETATION OF RESULTS

Class | Color Intensity | Interpretation
--- | --- | ---
- | No colored line appears | Negative
+ | Faint colored line | Low concentration of Allergen Specific IgE Antibody
++ | Distinct colored line | Medium concentration of Allergen Specific IgE Antibody
+++ | Strong colored line | High concentration of Allergen Specific IgE Antibody

1. A negative result indicates that the patient is non-atopic.
2. A positive result indicates that the patient may be atopic to this allergen.
3. The higher concentration of Allergen-Specific IgE Antibody is, the higher degree the patient is suffering the atopic.

Class Total IgE Color Intensity Interpretation
--- | --- | ---
- | No colored line appears | The concentration of Total IgE is less than the detection limit
+ | Faint colored line | Low concentration of Total IgE antibody
++ | Distinct colored line | Medium concentration of Total IgE antibody
+++ | Strong colored line | High concentration of Total IgE antibody

1. Result +++ is positive which indicates the patient may be atopic.
2. The Total IgE test of this kit cannot replace the single, quantitative ELISA test system.

NOTE: The test results are considered invalid if the below validation requirements are not met.

LIMITATIONS

1. Sensitivity: 0.35 IU / mL
2. Specificity: the rate of the cross-reactivity of the human IgA, IgG and IgM, etc. <0.021%
3. Precision: Intra to repetitive >99.9%

PERFORMANCE CHARACTERISTICS

1. Sensitivity: 0.35 IU / mL
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BIBLIOGRAPHY