**Human Allergen Specific IgE ELISA Kits**

**Intended Use**

These kits are *in vitro* assays for the qualitative or quantitative detection of allergen-specific IgE antibodies in human serum. They are intended for *in vitro* diagnostic use as an aid in the clinical diagnosis of IgE mediated allergic disorders in conjunction with other clinical findings.

**Principle**

The kits are designed in the format of indirect Enzyme-Linked Immunosorbent Assay (ELISA). IgE antibodies against an allergen which are present in human serum will bind onto the well of a microtitre plate (strip) pre-coated with the allergen. The bound IgE antibodies are detected by using horseradish peroxidase (HRP) conjugated antibody against human IgE, which in turn is visualised by adding 3,3’,5,5’-Tetramethylbenzidine (TMB) substrate solution. Any coloured product is measured at 450 nm after adding stopping solution. The colour intensity is proportional to the concentration of the specific human IgE antibodies in the serum.

**Kit Components Supplied**

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Allergens pre-coated microtiter plate (8 break apart strips 12 wells each)</td>
<td>1 × 96 well</td>
</tr>
<tr>
<td>2</td>
<td>HRP conjugate (ready to use)</td>
<td>1 × 6 ml</td>
</tr>
<tr>
<td>3</td>
<td>Sample diluents (ready to use)</td>
<td>1 × 5 ml</td>
</tr>
<tr>
<td>4</td>
<td>Wash buffer (30 × concentrate)</td>
<td>1 × 30 ml</td>
</tr>
<tr>
<td>5</td>
<td>TMB solution (ready-to-use)</td>
<td>1 × 12 ml</td>
</tr>
<tr>
<td>6</td>
<td>Stop solution (ready-to-use)</td>
<td>1 × 12 ml</td>
</tr>
<tr>
<td>7</td>
<td>Negative control (ready to use)</td>
<td>1 × 1.2 ml</td>
</tr>
<tr>
<td>8</td>
<td>Positive control (ready to use)</td>
<td>1 × 1.2 ml</td>
</tr>
<tr>
<td>9</td>
<td>Calibrator (100 IU/ml)</td>
<td>1 × 0.3 ml</td>
</tr>
</tbody>
</table>

**Shelf Life and Storage**

The shelf-life is 12 months from manufacture. The expiration date for the kits and each component is stated on the labels. Store at +2 - 8°C. Do not freeze.

**Samples**

1. Fresh serum is recommended. Avoid using bacteria-contaminated, lipemic, haemolysed or jaundiced specimen.
2. Serum should not be kept for more than 8 hours at room temperature (18-25°C), or over 48 hours at 2-8°C. For long-term storage, serum can be kept at -20°C. To avoid unreliable results caused by repeated freezing and thawing, serum should be stored in small aliquots without preservative.
3. All human fluids should be treated as infectious agents that could carry HIV.
**Operation Procedures**

Take all components of the kits and test serum samples from 2-8°C and leave for 30 minutes at room temperature (18-25°C) before testing. Re-pack all unused ELISA strips in the original aluminium pouch and put back to the storage fridge.

1. **Reagent Preparation:**
   1) **Wash Buffer**

   Based on the volume required, dilute the wash buffer concentrate by 1/30 with distilled or deionised water. The diluted wash buffer should be used within one month.

   2) **Sample**

   In general, it is not necessary to dilute the serum samples for the assay. If a sample gives a high OD (Optical Density) reading of > 3.00, dilute the sample 5 times with sample diluent and repeat the assay procedure.

   3) **Calibrator**

   The top human IgE calibrator is supplied at a concentration of 100 IU/ml. The calibrator dilution series should be prepared immediately prior to use as shown in the Table below. Mix well between each step.

<table>
<thead>
<tr>
<th>Calibrator Dilution</th>
<th>Concentration of Human IgE (IU/ml)</th>
<th>Volume of Human IgE Calibrator (ml)</th>
<th>Volume of Sample Diluent (ml)</th>
<th>Total Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>0.300</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>0.125 of dilution 1</td>
<td>0.125</td>
<td>0.250</td>
</tr>
<tr>
<td>3</td>
<td>17.5</td>
<td>0.100 of dilution 2</td>
<td>0.185</td>
<td>0.285</td>
</tr>
<tr>
<td>4</td>
<td>3.5</td>
<td>0.100 of dilution 3</td>
<td>0.400</td>
<td>0.500</td>
</tr>
<tr>
<td>5</td>
<td>0.7</td>
<td>0.100 of dilution 4</td>
<td>0.400</td>
<td>0.500</td>
</tr>
<tr>
<td>6</td>
<td>0.35</td>
<td>0.100 of dilution 5</td>
<td>0.100</td>
<td>0.200</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>Negative control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. **Assay Procedure**

   1) Once all reagents reach room temperature (18-25°C), secure the strips onto the supplied plate frame.

   2) For a qualitative assay, add 50 µl of positive IgE control and 50 µl of negative IgE control into the specified wells. For a quantitative assay, add 50 µl of each IgE calibrator dilution to the relevant wells. In this case, the positive control is not necessary.

   3) To the wells coated with each specific allergen, add 50 µl of serum per well.

   4) Mix well and incubate for 45min at 37°C.

   5) Discard all the reaction solution from the wells and wash five times with the diluted wash buffer. Blot plate onto paper towels or other absorbent material. For automated washing, aspirate plate contents from all wells and fill with wash buffer. Repeat procedure four additional times for a total of five washes.

   6) To each well, add 50 µl of HRP conjugate solution and incubate for 45 minutes at 37°C.

   7) Repeat the wash procedure as per step 5).
8) To each well, add 100 µl of TMB substrate and incubate at room temperature for 10-20 minutes.
9) To each well, add 100 µl of stop solution.
10) Read result with an ELISA reader at 450nm wavelength and record the OD_{450nm} readings.

Results Calculation

It is recommended to use validated software, such as Logistic four parameters model, for the calculation of the results. For manual calculation, the mean OD_{450nm} readings are calculated from the Calibrators and Controls. Generate a graph from the mean OD_{450nm} readings of the 6 Calibrators on half logarithmic paper (Abscissa: log IgE concentration at IU/ml; Ordinate: mean OD_{450nm} readings to create a calibration curve. The IgE concentration of tested patient serum is determined on the basis of this calibration curve.

Interpretation of Results

1. Test Validity
   1) The OD_{450nm} readings of negative control should be < 0.10
   2) The OD_{450nm} readings of positive control should be ≥ 1.0

2. Negative and positive determination:
   1) OD_{450nm} readings of test sample less than 0.150: Negative.
   2) OD_{450nm} readings of test sample more than 0.165: Positive.

3. The relationship between the concentrations of specific IgE and the grade of allergy is illustrated in the table below.

<table>
<thead>
<tr>
<th>Specific IgE concentration (IU/ml)</th>
<th>Grade of Allergy</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.35</td>
<td>0</td>
</tr>
<tr>
<td>0.35-0.70</td>
<td>1</td>
</tr>
<tr>
<td>0.71-3.50</td>
<td>2</td>
</tr>
<tr>
<td>3.51-17.50</td>
<td>3</td>
</tr>
<tr>
<td>17.51-50.0</td>
<td>4</td>
</tr>
<tr>
<td>50.01-100.0</td>
<td>5</td>
</tr>
<tr>
<td>&gt;100.0</td>
<td>6</td>
</tr>
</tbody>
</table>

Note: Total IgE level of a healthy adult person is generally less than 100IU/ml.

Performance of Assay

1) Sensitivity: ≤0.30 IU/ml.
2) Precision: The inter-assay CV% is ≤10% and the intra-assay CV% is ≤15%
3) Accuracy: Recovery rate is in the range of 90.0% -110.0%
4) Specificity: Cross-reactivity with human IgA, IgG and IgM is ≤0.35 IU/ml.

Limitations

For in vitro diagnostic use only.
No single assay should be used as the basis for arriving at a diagnostic conclusion.
Test Panels

Inhalant Allergens Panel

Product Number: IA-IgE - ELISA -001
Package Size: 96 tests (12x8 strips)
Allergens included:
- Dermatophagoides pteronyssinus
- Dermatophagoides farinae
- House dust
- Roach
- Cat Hair
- Dog Hair
- Herba plantaginis
- Asiaticwormwood
- Molds (Penicillium chrysogenum, Aspergillus fumigates, Alternaria alternate, Cladosporium herbarum)
- Tree pollens (oak, elm, sycamore mix, willow, poplar EM).

Food Allergens Panel

Product Number: FA-IgE - ELISA -001
Package Size: 96 tests (12x8 strips)
Allergens include:
- Milk
- Egg White
- Beef
- Lamb
- Salmon
- Shrimp
- Crab
- Cashew nut
- Peanut
- Mango

Mixed Allergens Panel

Product Number: MA-IgE - ELISA -001
Package Size: 96 tests (12x8 strips)
Allergens include:
- Dermatophagoides pteronyssinus
- House dust
- Dog Hair
- Roach
- Milk
- Egg White
- Shrimp
- Crab
- Molds (Penicillium chrysogenum, Aspergillus fumigates, Alternaria alternate, Cladosporium herbarum)
- Tree pollens (oak, elm, sycamore mix, willow, poplar EM).

NOTE:

If the allergen that you need to test is not listed above, or the allergens listed above are not what you need, please do not hesitate to contact us. We will do our best to meet your requirements.