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# Product Information

## IgE ELISA Kit

Catalog Number: EA100989
Storage Temperature: 2 – 8°C

# Instruction for Use

**THIS KIT IS INTENDED FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

## 1 INTRODUCTION

### 1.1 Intended Use

The **ORIGENE IgE ELISA** is intended for the quantitative determination of Immunoglobulin E (IgE) in human serum.

## 2 PRINCIPLE OF THE TEST

The ORIGENE IgE ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA).
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) conjugation solution. The test specimen (serum) is added to the IgE antibody coated microtiter wells and incubated with the Zero 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 labelled 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 the enzyme-linked antibodies. A solution of TMB reagent is added and incubated at room temperature 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 REAGENTS

### 3.1 Reagents provided

1) ***Monoclonal Anti-IgE coated Microtiter plate with 96 wells***
2) ***Zero Buffer***, 13 ml
3) ***Enzyme Conjugate Reagent,*** *18 ml*
4) ***IgE Reference Standards***, 6 vials, 0.5 ml each;
   Concentrations: 0 – 10 – 50 – 100 – 400 – 800 IU/ml (WHO, 2nd IRP, 75/502)
5) ***TMB Reagent***, 11 ml
6) ***Stop Solution***, (1 N HCl), 11 ml

### 3.2 Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm)
- Vortex mixer or equivalent
- Calibrated variable precision micropipettes and tips (20 µl, 100 µl, 150 µl).
- Absorbent paper.
- Deionized water
- Timer
- Graph paper or software for data reduction

### 3.3 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for 8 weeks if stored as described above.

### 3.4 Reagent Preparation

1. Bring all reagents and required number of strips to room temperature (18 – 25 °C) prior to use.
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Samples with expected values greater than 800 IU/mL should be diluted with Zero Standard prior to assaying. A 1:100 initial dilution is recommended.

## 4 ASSAY PROCEDURE

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **20 µL** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3. Dispense **100 µL *of Zero Buffer*** into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing in this setup.
5. Incubate for **30 minutes** at room temperature (18 – 25 °C).
6. Remove the incubation mixture by flicking plate content into the waste container.
7. Rinse and flick the microtiter plate **5 times** with **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 *Enzyme Conjugate Reagent*** into each well. Gently mix for 10 seconds.
10. Incubate for **30 minutes** at room temperature.
11. Remove the incubation mixture by flicking plate content into the waste container.
12. Rinse and flick the microtiter plate 5 times with deionized water (Please do not use tap water).
13. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
14. Dispense **100 µL** of ***TMB Reagent*** to each well. Gently mix for 10 seconds.
15. Incubate for **20 minutes** at room temperature in the dark.
16. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
17. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
18. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.

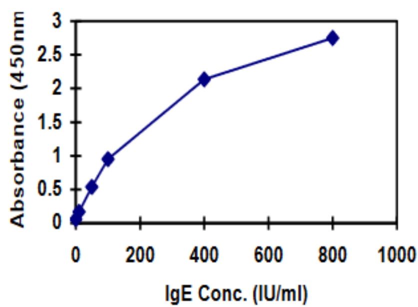
### 4.1 Calculation of Results

1. Calculate the means absorbance values (A450) for each set of standards, controls and samples.
2. Construct the standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in IU/ml on linear graph paper, with absorbance values on the vertical (y) axis and concentrations 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.
4. Sample Dilution: If a sample contains more than 800 IU/mL of IgE, make a 1:100 dilution or further dilutions with the zero standard. After assaying the diluted sample, multiply the calculated value by the appropriate dilution factor.
5. Any diluted samples must be further converted by the appropriate dilution factor.

#### 4.1.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

| Standard | Optical Units (450 nm) |
|---|---|
| Standard 0 (0 U/mL) | 0.058 |
| Standard 1 (10 U/mL) | 0.167 |
| Standard 2 (50 U/mL) | 0.538 |
| Standard 3 (100 U/mL) | 0.950 |
| Standard 4 (400 U/mL) | 2.135 |
| Standard 5 (600 U/mL) | 2.748 |

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