



## **IgE Elisa Kit**

### **Enzyme Immunoassay for Quantitative Determination of immunoglobulin E (IgE) in Human Serum**

#### **1. INTENDED USE**

IgE Kit is intended for the quantitative determination of immunoglobulin E(IgE) in human serum

Normally IgE concentration in serum is very low. It increases gradually from birth to teen-age. In adults normal concentration of IgE may reach 100 IU/ml . In elderly people IgE level some times decreases.IgE production is essential in anti-helmenthic immunity. 15-20-fold increase in IgE concentration (up to 1500-2000IU/ml)is observed in tha case of ascariasis .But in industrialized countries detection of high IgE concentrations is mainly connected with allergic diseases.Quantitative determination of total IgE has a great prognostic value . in 75% of children born from parents with allergic diseases serum IgE concentration in serum by enzyme immunoassay is an important tool for differentiation between allergic diseases and other pathologies with similar clinical manifestations (such as asthma ,frequent respiratory diseases ,chronic rhinitis and dermatitis).

Increased concentration of total IgE in serum was also reported in patients with lymphosarcoma and hyper-IgE syndrome.

#### **PRINCIPLE OF THE TEST**

Allergo EIA-IgE is a "sandwich" type of solid –phase enzyme immunoassay .based on two monoclonal antibodies that are specific for different epitopes of IgE molecule .One of these antibodies is conjugated with horseradish peroxidase, the other is immobilized on inner surface of microwells .IgE molecules from the serum sample bind to both immobilized antibodies and anti-IgE peroxidase conjugate .Then the wells are washed with wash buffer to remove any material not bound to the inner surface of the wells .the quantity of the bound conjugate is in direct proportion to the IgE concentration in tested sample .During the incubation with TMB substrate solution the coloring appears .The color intensity is in direct proportion to the IgE concentration in sample .The enzyme reaction is stopped by dispensing the acidic solution (1N HCL)into the wells .Optical density of the solution in the wells is directly proportional to the IgE concentration in the samples .The standard curve is plotted by using the IgE concentrations in the calibrators (x-axis) and their corresponding OD values (y-axis) .The IgE concentration of the specimen is directly read off from the standard curve .

If expected IgE concentrations in the samples are higher than in Calibrator 5. the samples should be diluted 20- fold with the Calibrator 0(0U/ml).In this way .higher IgE concentrations become accessible.

#### **PATINTS SAMPLES**

**Specimen collection and storage:**

Blood is taken aseptically by venipuncture .After clotting,the serum is separated by centrifugation .DO not use plasma .hemolysed or lipemic serum and serum with sodium azide added as preservative .

Store serum samples at +2.....8C for no more that 2 days. for longer storage it is recommended to aliquote and freeze at -20 or below. Avoid repeated freezing .

## **TEST COMPONENTS**

- **microtiter plate**:12x8 wells coated with anti-IgE monoclonal antibodies.
- **concentrated wash buffer**.
- **conjugate**: contains anti-IgE antibodies conjugated with HPR .
- **TMB substrate** : 3,3',5,5' – tetramethylbenzidine solution in citrate buffer containing hydrogen peroxide.
- **stop reagent**:1N HCL solution
- **IgE calibrators**(protein-based buffer containing known IgE concentration), see vial's label.
- **IgE control**( protein-based buffer containing known IgE concentrations), see vial's label.

## **Preparation before use:**

Preior to assay,allow the samples to reach room temperature. Take care to agitate serum samples gently in order ensure homogeneity.

## **MATERIAL REQUIRED BUT NOT SUPPLIED WITH THE KIT:**

-A set of digital variable pipettes that cover volume range from 5µl . with appropriated disposable Tips.

-8-channel digital variable pipette covers volume range up to 0.3ml with appropriate disposable

Tips.

-microplate shaker thermostat, able to maintain temperature +37°C and shaking speed 500 to 800rpm.

-microplate washer or 8-chnnel wash comb with vacuum pump and waste bottle:

-automatic microplate reader with optical filter for 450 nm

-volumetric cylinder

-volumetric beaker

-distilled or deionzed water

## **SIZE AND STORAGE**

IgE kit is designed for 96 determination. This is sufficient for 40 unknowns, 6 calibrators, 1 control and 1 TMB substrate control in duplicates, provided that all the strips are used simultaneously.

Please take into consideration that calibrators should be measured in each separate assay. It is also recommended to measure IgE concentration in the control each time.

NOTE: if used partially, kit should be utilized within a month after opening.

The expiry date of the kit is reported on the box label, expiry date for each component is indicated on the respective label.

Upon receipt, AllergoEIA-Totral IgE kit should be stored at 2-8°C, preferably in the original kit box.

If used for separate experiments, kit contents should be stored as follows:

- the unused strips : in a firmly closed plastic bag at 2-8 °C until expiry date.
- opened vials with conjugate and TMB substrate solution :at 2-8°C for no more than 1 month.
- concentrated wash buffer:at 2-8°C until expiry date.
- wash buffer prepared for use: in a firmly closed bottle for no more than 5 days at room temperature.
- calibrators and controls: at 2-8°C for no more than 1 month after opening.
- stop reagent at 2-8°C until expiry date.

## PREPARATION BEFORE USE

Before the assay, allow all the kit components to reach a room temperature and stir thoroughly .

**A.** the microtitration plat consists of a frame and breakable strip .packed in the foil bag .Before opening .Keep the bag at room temperature (+18...+25°C)for at least 30minutes .Open the bag and place required number of strips on strip holder .put remaining strip back in the original plastic bag with ziplock closure and close firmly ..keep at +2...+8°C until expiry date stated on the label .

**B-**prepare the necessary volume of wash buffer by dilution the concentrate 20-fold with distilled or deionized water .for example:

5ml of concentrate +95ml of distilled water

Mix thoroughly .avoiding foaming .keep the prepared wash buffer firmly closed .store at room temperature (+18...25°C)for no more than 5 days

The rest of the concentrated wash buffer should be stored firmly closed at+2...8°C until expiry date .

C. IgE calibrators and control are ready for use .Once opened .store at +2...+8°C for no more than 1 month.

.E-protect the TMB substrate solution from direct light

## ASSAY PROCEDURE

All the sample should be tested in duplicates

1. Bring all the reagents to room temperature before use .Stir gently .without causing foaming
2. Dispense 150ul of Conjugate into each well. Except A1-A2 wells
3. Dispense

20 µl of IgE calibrators(0 to 5):

20 µl of IgE control:

20 µl of patients sample

Into the respective wells.

**Note :Total time of dispensing must not exceed 15 minutes .otherwise the test result may be unreliable . because the time of incubation with conjugate will substantially vary for different samples**

4. Incubate strips for 1.5 hours while shaking (500-800) rpm at +37 °C
5. Decant . then wash each well 5 times with 300 µl of wash buffer .Make sure that after the last washing cycle the residual buffer is thoroughly aspirated from the wells
6. Immediately add 100µl of TMB substrate solution into each well
7. Incubate for **15- 30minutes at room temperature in the dark** .depending on the color intensity
8. add 100 µl of stop reagent to all the well and shake well for 1-2 minutes
9. Read the optical density at a wavelength of 450 nm

## DATA PROCESSING

Data processing is done by a computer assisted analysis by plotting the mean OD values of the calibrators at 450 nm versus their respective IgE concentrations using 4PL or 5PL fit. Mean OD of the A1-A2 wells is used as blank.

Any extrapolation of the standard curve to IgE concentration above the nominal value of calibrator 5 (approximately 500 U/ml) is not permitted. In this case the sample should be diluted 20 fold with calibrator 0 and re-tested.

## REFERENCE VALUES

Total IgE concentration in serum samples collected at 9-11 a.m. from 79 healthy individuals (age 21-45) and 226 patients with allergic diseases (of the same age) was measured with "EIA- total IgE" assay. Among healthy individuals in 48% cases IgE concentration was below 25 IU/ml; in 34% cases – between 25- 100 IU/ml, and only in 18% cases – above 100 IU/ml.

Among allergic patients IgE concentration below 25 IU/ml was detected in 8% cases; between 25-100 IU/ml – in 22% cases and above 100 IU/ml – in 70% cases. These limits should be considered as guidelines only.

It is highly recommended for each laboratory to determine its own reference range of IgE concentrations.

## PERFORMANCE CHARACTERISTICS OF THA ASSAY

- **calibration:** AllergoEIA-IgE test kit is calibrated against WHO 2<sup>nd</sup> international reference preparation 75/502.

- **units of measure:** concentrations of total IgE in calibrators are expressed in IU/ml. to convert into ng/ml, multiply the concentration in IU/ml by 2.4.

- **dilution parallelism of serum samples:** serial dilutions of three human serum samples with predetermined concentration of total IgE in calibrator 0 were assayed with IgE kit with the following results:

sample	dilution	Measured concentration, nmol/l	Expected concentration, nmol/l	Observed/ expected concentration ratio,%
1	undiluted	557.5		
	1:2	252	278.8	90.4%
	1:4	126.3	139.4	91.3%
	1:8	61.7	69.7	88.6%
	1:16	31.5	34.8	90.4%
2	undiluted	386		
	1:2	179.1	193	92.2%
	1:4	93.1	96.5	97.1%
	1:8	46.9	48.3	96.5%
	1:16	22.2	24.1	92.8%
3	undiluted	170.6		
	1:2	82.6	85.3	90.7%
	1:4	39.3	42.7	95.8%
	1:8	20.4	21.3	92.1%
	1:16	9.8	10.7	96.8%

- **Specificity:** no cross reactivity was detected between anti- IgE monoclonal antibodies used in the assay and IgG, IgM and IgA.

For IgE test kit no **high dose hook effect** was detected for IgE concentrations up to 10000 IU/ml.

**Analytical sensitivity(lower detection limit):** analytical sensitivity of IgE assay, or the lowest detectable concentration that can be distinguished from zero calibrator, is 5 IU/ml. it is defined as mean OD of 10 replicates of calibrator 0 plus 2 standard deviations.

**LIMITATION OF THE METHOD** any clinical diagnosis should not be based in the results of in vitro diagnostic method alone. To state a diagnosis, the physician is supposed to consider all the available clinical and laboratory findings.

## SAFETY PRECAUTIONS

- this kit is for invitro diagnostic use only. The operator should be thoroughly follow the manual to obtain the reliable data. This instruction manual is valid only for the present kit with the listed composition. Any exchange of kit components is not allowed by CE regulations.
- do not use kits or components after expiry date stated on the label. take into consideration stability period for reconstituted reagents.
- do not mix or use together reagents of different lots.
- stop reagent is 1N HCL solution. Avoid contacts with skin and mucosa. In case of contact rinse affected region thoroughly with plenty of water and seek medical advice.
- source materials of human origin that were used in preparation of kit were tested and found negative for HBsAg, anti-HIV and anti-HCV antibodies. How ever, no known laboratory test guarantees the absence of these viral agents. Therefore, all the kit components and patient's samples should be handled as potentially hazardous.
- As the kit contains potentially hazardous material, the following precautions should be observed:
  - \*Do not smoke, eat or drink while performing the assay.
  - \*Always use protective gloves.
  - \*Never pipette material by mouth.
  - \*In the case of spilling, wipe up the spills promptly and wash the affected area thoroughly with decontaminant.
- GLP and all general and individual regulations should be applied to the use of the kit.

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