## Instructions for Use

# Immunoglobulin E (IgE) ELISA

IVD

CE

REF **EIA-1788**

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***Please use only the valid version of the Instructions for Use provided with the kit.***

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**FOR IN VITRO DIAGNOSTIC USE**

Store at 2 °C to 8 °C.

## 1 INTENDED USE

The DRG IgE ELISA is intended for the quantitative determination of immunoglobulin E in human serum.

This assay is to be used for the assessment of hypersensitive and allergic reactions in patients.

## 2 INTRODUCTION

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.[1,2,3] IgE is also known as the reaginic 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.[4,5] Decreased levels of IgE are found in cases of hypogammaglobulinemia, autoimmune diseases, ulcerative colitis, hepatitis, cancer, and malaria.[6] Cord blood or serum IgE levels may have prognostic value in assessing the risk of future allergic conditions in children.[7,8]

Certain groups of white blood cells, including basophils and tissue mast cells, have membrane receptors for the IgE molecule. These target cells, through a series of complex reactions, form a combination of a specific allergen with antibody-sensitized basophils or mast cells, and initiate the release of certain vasoactive agents, such as histamine, into the blood stream.[9,10,11] As a result, there is a constriction of smooth muscles, dilation of small blood vessels, activation of blood platelets, and irritation of skin nerve endings characteristic of allergic reactions. Typical clinical symptoms of immediate hypersensitivity are inflammation and itching in a skin reaction, or congestion in a bronchial reaction.

The IgE serum concentration in a patient is dependent on both the extent of the allergic reaction and the number of different allergens to which the patient is sensitized. Non-allergic normal individuals have IgE concentrations that vary widely and increase steadily during childhood, reaching their highest levels at age 15 to 20, and thereafter remaining constant until about age 60, when they slowly decline.[12]

## 3 PRINCIPLE OF THE ASSAY

The DRG IgE Quantitative Test is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.[13,14] The test specimen (serum) is added to the IgE monoclonal antibodies immobilized on polystyrene microtiter wells (solid phase) and incubated with the Zero Buffer. If human IgE is present in the specimen, it will combine with the antibodies on the well. The well is then washed to remove any residual test specimen, and goat anti-IgE in the antibody-enzyme (horseradish peroxidase) conjugate reagent is added. The conjugate reagent 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. After incubation at room temperature, the solid phase is washed with water to remove unbound labeled antibody. A solution of 3,3',5,5'-Tetramethylbenzidine (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped and the resulting yellow color is measured spectrophotometrically at 450 nm. The concentration of IgE is directly proportional to the color intensity of the test sample.

The DRG IgE ELISA provides a rapid, sensitive, and reliable assay for total serum IgE. Two carefully selected IgE antibodies are used to determine a minimal concentration of IgE of 5.0 IU/mL.

## 4 REAGENTS AND MATERIALS PROVIDED

1. **Antibody-Coated Wells** (1 plate, 96 wells)
   Microtiter wells coated with mouse monoclonal anti-IgE.
2. **IgE Zero Buffer** (13 mL)
   Contains Tris buffer with yellow dye and preservative.
3. **Enzyme Conjugate** Reagent (18 mL)
   Contains bovine serum with red dye and preservative.
4. Reference **Standard** Set (0.5 mL/vial)
   Contains 0, 10, 50, 100, 400, and 800 IU/mL (WHO, 2nd IRP, 75/502) of Immunoglobulin E in bovine serum with preservative.
   Liquid, ready to use.
5. **TMB Reagent** (1 bottle, 11 mL)
   Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
6. **Stop Solution** (1N HCl) (1 bottle, 11 mL)
   Contains diluted hydrochloric acid.

## 5 MATERIALS REQUIRED BUT NOT PROVIDED

1. Deionized water
2. Precision pipettes: 0.02, 0.1, 0.15, 0.2 and 1 mL
3. Disposable pipette tips
4. Microwell reader capable of reading absorbance at 450 nm.
5. Vortex mixer, or equivalent
6. Absorbent paper
7. Linear Graph paper
8. Quality control material (e.g., BioRad Lyphochek Control sera)

## 6 WARNINGS AND PRECAUTIONS

1. CAUTION: This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
10. For in vitro diagnostic use.

## 7 STORAGE CONDITIONS

1. Store the unopened kit at 2 °C to 8 °C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. The opened and used reagents are stable until the expiration date if stored properly at 2 °C to 8 °C.
3. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

## 8 SPECIMEN COLLECTION AND PREPARATION

1. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques.
   This kit is for use with serum samples without additives only.
   Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples.
2. Specimens should be capped and may be stored for up to 48 hours at 2 °C to 8 °C.
   Specimens held for a longer time should be frozen only once at -20 °C prior to assay.
   Thawed samples should be inverted several times prior to testing.

## 9 INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 2 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

## 10 REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18 °C to 25 °C) before 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.

## 11 ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense **20 µL** of **standards, samples, and controls** into appropriate wells.
3. Dispense **100 µL** of **Zero Buffer** into each well.
4. Thoroughly mix for 10 seconds. It is very important to have complete mixing in this setup.
5. Incubate at room temperature (18 °C to 25 °C) for 30 minutes.
6. Remove the incubation mixture by flicking plate content into a 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** of **Enzyme Conjugate** Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature (18 °C to 25 °C) for 30 minutes.
11. Remove the incubation mixture by flicking well contents into a suitable waste container.
12. Rinse the wells 5 times with running deionized water. (Please do not use tap water.)
13. Strike the wells sharply on absorbent paper to remove residual water droplets.
14. Dispense **100 µL TMB Substrate Reagent** into each well. Gently mix for 10 seconds.
15. Incubate at room temperature, in the dark, for 20 minutes.
16. Stop the reaction by adding **100 µL** of **Stop Solution** (1N HCl) into 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 OD at 450 nm with a microtiter well reader **within 15 minutes**.

## 12 CALCULATION OF RESULTS

1. Calculate the mean absorbance value (OD450) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in IU/mL on graph paper, with absorbance on the vertical (y) axis and concentration 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. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
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.

## 13 STANDARDIZATION

The Reference Standards are standardized against the World Health Organization Reference 2nd IRP, 75/502 (1981).

## 14 PROCEDURAL NOTES

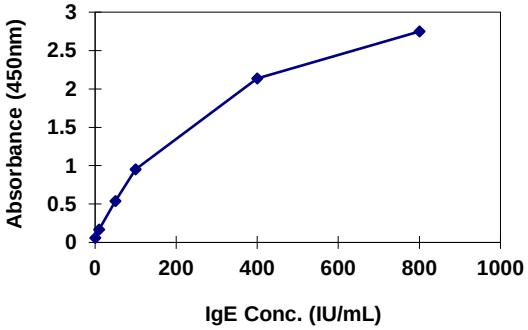
1. *Manual Pipetting:*
   It is recommended that no more than 32 wells be used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. *Automated Pipetting:*
   A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes.
3. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
4. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

## 15 EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the y-axis against IgE concentrations shown in the x-axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own standard curve and patient data in each experiment.

| IgE (IU/mL) | Absorbance (450 nm) |
|---|---|
| 0 | 0.058 |
| 10 | 0.167 |
| 50 | 0.538 |
| 100 | 0.950 |
| 400 | 2.135 |
| 800 | 2.748 |

This standard curve covers a dynamic range from 5 to 800 IU/mL IgE. It is for illustration only, and should not be used to calculate unknowns.




## 16 EXPECTED VALUES

The total Immunoglobulin E level in normal, allergy-free adults is less than 150 IU/mL in the serum. Variation in total IgE concentrations may be expected in certain age groups and clinical conditions, as briefly described in the “Introduction” above.

Each laboratory should establish its own normal ranges based on patient population in the geographical areas served. These values have clinical significance only after a statistically significant number of assays have been performed over a suitable period of time.

The available literature provides the following information relative to total Immunoglobulin E in human serum:[12,16,17,18,19]

Normal geometric mean: 39 IU/mL IgE

Normal ranges:

| Age (years) | IgE (IU/mL) |
|---|---|
| 1 - 5 | < 60 |
| 6 - 9 | < 90 |
| 10 - 16 | < 200 |
| 16 + | < 100 |

## 17 PERFORMANCE CHARACTERISTICS

### 17.1 Accuracy

A statistical study using 98 healthy patient samples, ranging in IgE concentration from 6.4 IU/mL to 1039.2 IU/mL, demonstrated good correlation with a commercially available kit (Hybritech Tandem™-E IgE) as shown below.

N = 98

Correlation coefficient = 0.96

Slope = 1.041

Intercept = -23.77

DRG Mean = 209.4 IU/mL

Bio-Rad Mean = 210.1 IU/mL

### 17.2 Sensitivity

The minimum detectable concentration of the DRG IgE ELISA assay as measured by 2SD from the mean of a zero standard is estimated to be at least 5.0 IU/mL.

### 17.3 Precision

#### a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of three different serum samples in one assay.

Within-assay variability is shown below:

| Serum Sample | 1 | 2 | 3 |
|---|---|---|---|
| Number of Replicates | 24 | 24 | 24 |
| Mean IgE (IU/mL) | 339.5 | 171.8 | 105.1 |
| Standard Deviation | 18.6 | 11.5 | 4.8 |
| Coefficient of Variation (%) | 5.5% | 6.7% | 4.6% |

#### b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of three different serum samples over a series of individually calibrated assays.

Between-assay variability is shown below:

| Serum Sample | 1 | 2 | 3 |
|---|---|---|---|
| Number of Replicates | 24 | 24 | 24 |
| Mean IgE (IU/mL) | 384.4 | 172.4 | 91.9 |
| Standard Deviation | 20.8 | 12.5 | 4.9 |
| Coefficient of Variation (%) | 5.4% | 7.3% | 5.3% |

### 17.4 Hook Effect

No hook effect was observed in this test, since it is a sequential assay procedure.

### 17.5 Recovery and Linearity Studies

#### a. Recovery

Various patient serum samples of known IgE levels were combined and assayed in duplicate. The mean recovery was 98.0%.

| Expected Concentration (IU/mL) | Observed Concentration (IU/mL) | % Recovery |
|---|---|---|
| 489.3 | 481.9 | 98.5 |
| 301.5 | 309.1 | 102.5 |
| 208.5 | 181.8 | 87.2 |
| 86.3 | 82.2 | 95.3 |
| 49.3 | 48.0 | 97.3 |
| 27.8 | 27.4 | 98.4 |
| 14.7 | 14.3 | 97.6 |
| 542.5 | 533.2 | 98.3 |
| 313.7 | 296.4 | 94.5 |
| 132.6 | 129.9 | 97.9 |
| 68.7 | 68.9 | 100.3 |
| 22.5 | 21.4 | 95.1 |

#### b. Linearity

Three patient samples were serially diluted to determine linearity. The mean recovery was 103.7%.

| # | Dilution | Expected Conc. (IU/mL) | Observed Conc. (IU/mL) | % Expected |
|---|---|---|---|---|
| 1. | Undiluted | -- | 1000 | -- |
| | 1:2 | 500 | 576.7 | 115.3% |
| | 1:4 | 250 | 249.1 | 99.6% |
| | 1:8 | 125 | 126.6 | 101.3% |
| | 1:16 | 62.5 | 66.8 | 107.2% |
| | 1:32 | 31.3 | 36.6 | 116.9% |
| | 1:64 | 15.6 | 16.8 | 107.7% |
| 2. | Undiluted | -- | 704.1 | -- |
| | 1:2 | 352.1 | 332.5 | 94.4% |
| | 1:4 | 176.0 | 159.8 | 90.8% |
| | 1:8 | 88.0 | 76.3 | 86.7% |
| | 1:16 | 44.0 | 42.2 | 95.9% |
| | 1:32 | 22.0 | 23.2 | 105.5% |
| | 1:64 | 11.0 | 10.8 | 98.2% |
| 3. | Undiluted | -- | >1000 | -- |
| | 1:2 | -- | 978.5 | -- |
| | 1:4 | 489.3 | 443.4 | 90.6% |
| | 1:8 | 244.6 | 223.2 | 91.3% |
| | 1:16 | 122.3 | 111.5 | 91.2% |
| | 1:32 | 61.2 | 56.5 | 92.3% |
| | 1:64 | 30.6 | 31.6 | 103.3% |
| 3. | Undiluted | -- | 800.8 | -- |
| | 1:2 | 400.4 | 425.3 | 106.2% |
| | 1:4 | 200.2 | 207.4 | 103.6% |
| | 1:8 | 100.1 | 94.9 | 94.8% |
| | 1:16 | 50.1 | 51.4 | 102.6% |
| | 1:32 | 25.0 | 29.1 | 116.4% |
| | 1:64 | 12.5 | 14.7 | 116.4% |

### 17.6 Specificity

The following human immunoglobulins were tested for cross reactivity:

| Immunoglobulin Tested | Concentration | Produced Color Intensity Equal to IgE in Serum (IUmL) |
|---|---|---|
| Human Immunoglobulin A | 400 mg/dL | < 5 IU/mL |
| Human Immunoglobulin G | 400 mg/dL | < 5 IU/mL |
| Human Immunoglobulin M | 400 mg/dL | < 5 IU/mL |

## 18 QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges.

## 19 LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. For professional use only. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician. IgE-mediated allergy should not be ruled out when test results are low or normal, or implicated as the single source when the test results indicate elevated IgE levels. There are patients with elevated serum IgE levels who are free of allergic symptoms, as well as patients with allergic problems who have normal serum IgE.
3. Slight hemolysis and/or lipemia do not interfere with the assay. The presence of sediment or precipitates in specimens or reagents may indicate contamination and should not be used.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. If the device fails to perform, use alternative diagnostic procedure or consult manufacturer.

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## SYMBOLS USED

| Symbol | English |
|---|---|
| CE | European Conformity |
|  | Consult instructions for use |
| IVD | *In vitro* diagnostic medical device |
| REF | Catalogue number |
| LOT | Batch code |
|  | Contains sufficient for <n> tests |
|  | Temperature limit |
|  | Use-by date |
|  | Manufacturer |
|  | Caution |
| | |
| RUO | For research use only |
| *Distributed by* | Distributed by |
| *Content* | Content |
| *Volume/No.* | Volume / No. |

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