Immunoglobulin E (IgE)

ENZYME IMMUNOASSAY TEST KIT

Enzyme Linked Immunosorbent Assay (ELISA) for Quantitative Determination of Immunoglobulin E (IgE) Concentration in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Immunoglobulin E (IgE) sandwich ELISA test is intended for the quantitative determination of Immunoglobulin E (IgE) Concentration in Human Serum For In Vitro Diagnostics Use only.

INTRODUCTION

IgE is also known as the reagenic 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. Decreased levels of IgE are found in cases of hypogammaglobulinemia, autoimmune diseases, ulcerative colitis, hepatitis, cancer, and malaria. Cord blood or serum IgE levels may have prognostic value in assessing the risk of future allergic conditions in children.

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 he is sensitized. Nonallergic normal individuals have IgE concentrations that vary widely and increase steadily during childhood, reaching their highest levels at age 15 to 20, and there after remaining constant until about age 60, when they slowly decline. 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. The IgE Quantitative Enzyme Immunoassay provides a rapid, sensitive, and reliable assay for total serum IgE.

PRINCIPLE OF THE ASSAY

The IgE Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-IgE antibody for solid phase (microtiter wells) immobilization and another anti-IgE antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the IgE antibody coated microwells and incubated with the Zero Buffer. 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 labeled with horseradish peroxidase (conjugate) is added. The conjugate will bind immunologically to the IgE on the well, resulting in the IgE molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a incubation at room temperature, the wells are washed with Wash Buffer to remove unbound labeled antibodies. A solution of TMB is added and incubated 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.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with anti- IgE antibody.
- Zero Buffer. Ready to use.
- Enzyme Conjugate. Ready to use.
- TMB Substrate. Ready to use.
- Stop Solution. Ready to use.
- IgE Standard set of 6 standards labeled as A to F in liquid form. Ready to use. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X).
- Control Set
- Pack Insert
- Plate Sealer
- Protocol Sheet
- Microwell Holder

Materials required but not provided

- Precision pipettes: 10µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

STORAGE AND STABILITY

1. **IgE** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated Microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the desiccant has changed from blue to pink at the time of opening the pouch, another coated Microwells pouch should be used.
3. Diluted Wash Buffer is stable for upto one week when stored at 2-8°C.

SPECIMEN COLLECTION

1. Collect Blood specimen by venipuncture according to standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

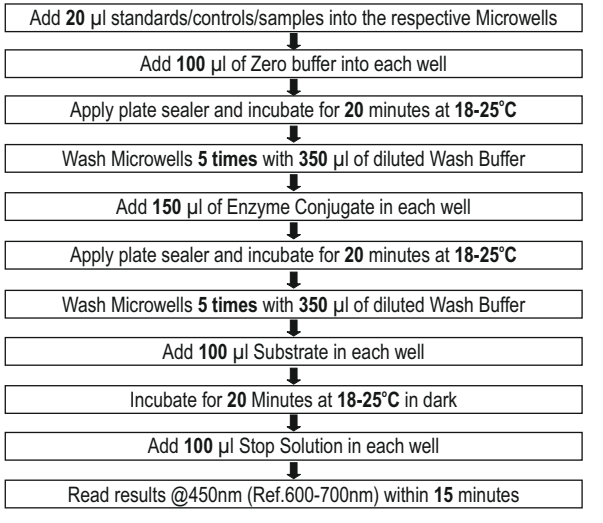
1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens, standards and controls should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense **20 µl** of standard, controls, specimens 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 (18-25°C) for **20 minutes**.
6. After incubation, empty the microtitre wells and wash the plate **5 times** with **350 µl** of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual droplets.
7. Dispense **150 µl** of Enzyme Conjugate into each well.
8. Incubate at room temperature for **20 minutes**.
9. After incubation, empty the microtitre wells and wash the plate **5 times** with **350 µl** of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual droplets.
10. Dispense **100 µl** TMB substrate into each well.
11. Incubate at room temperature in the dark for **20 minutes**.
12. Stop the reaction by adding **100 µl** of Stop Solution to each well.
13. Gently mix for **10 seconds**. It is important to make sure that all the blue color changes to yellow color completely.
14. Read optical density at 450/650 nm with a microtiter reader.



CALCULATION OF RESULTS

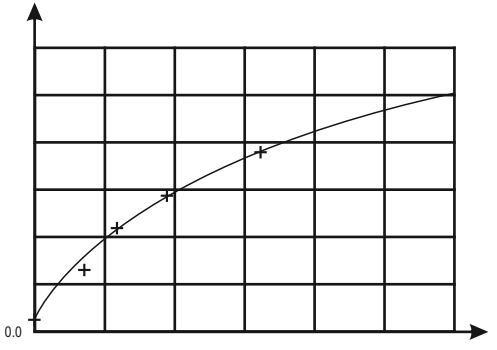
Calculate the mean absorbance value for each set of reference standards, specimens and patient samples. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in IU/ml on graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of IgE in IU/ml from the standard curve.

Example of Standard curve

Results of typical standard run with optical density reading at 450nm (ref 600-700nm) shown in the Y axis against IgE concentrations shown in the X axis.

Suggest: Use 4-Parameter Standard curve to calculate sample values.

IgE (IU/ml)	Absorbance (450nm)
A	0.0168
B	0.1588
C	0.5314
D	0.8995
E	1.6582
F	1.8180



This standard curve is for the purpose of illustration only, and should not be used to calculate samples. Each user should obtain his or her own standard curve and data.

Expected Ranges of values

Each laboratory must establish its own normal ranges based on patient population. Based on our internal evaluation data the following normal range is recommended:

Adults: < 300 IU/ml

The minimal detectable concentration of IgE by this assay is estimated to be 5 IU/ml.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. Accuracy: In an internal Study **IgE** was evaluated against commercially available licensed kit with 90 random clinical samples and **IgE** has demonstrated 95% clinical correlation with the commercially available licensed kit.
2. Precision: **IgE** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with **IgE**	Coefficient of Variation (CV)
Level 1	10	275.35	5.25
Level 2	10	110.29	5.18
Level 3	10	138.79	5.82

B) External Evaluation:

IgE ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **IgE** ELISA has demonstrated 95% correlation with the reference method.

*Data file: Orchid Biomedical Systems (P) Ltd.

IMPORTANT NOTE

1. The IgE assay is a temperature sensitive assay. The best temperature condition for this assay is from 18°C to 25°C.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
4. Duplication of standards, controls & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. The results obtained from this assay are not diagnostic proof of the presence or absence of a disease.
2. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

BIBLIOGRAPHY

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5. Kulczyski A. Jr. **J. Allergy Clin. Immunol**. 1981; 68: 5
6. Data on file: Orchid Biomedical Systems (P) Ltd.

SYMBOL KEYS

Temperature Limitation		Consult Instructions for use	
Manufacturer		IVD	*In vitro* Diagnostic Medical Device
Use by		REF	Catalogue Number
Date of Manufacture		LOT	Batch Number / Lot Number
This side up		Σ	Contains sufficient for <n> tests
Do not reuse			

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