Atlas Medical

ENZYME IMMUNOASSAY TEST KIT for Quantitative Determination of IMMUNOGLOBULINE (IgE) Concentration in Human Serum

IVD For *in vitro* diagnostic and professional use *only*

Store at 2 to 8°C.

96 Tests

INTENDED USE

For the quantitative determination of Immunoglobulin E (IgE) concentration in human serum.

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. 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. No 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. The IgE quantitative Enzyme Immunoassay provides a rapid, sensitive and reliable assay for total serum IgE. The minimal sensitivity of this assay is about 5.0 IU/mL.

PRINCIPLE OF THE TEST

The IgE Quantitative Test is based on 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) conjugate solution. The test specimen (serum) is added to the IgE antibody coated microtiter wells 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) 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 enzyme-linked antibodies. After incubation at room temperature, the wells are washed with water to remove unbound-labeled 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 2N HCL (Stop Solution), and the color is changed to yellow and measured spectrophotometrically at 450-630 nm. The concentration of IgE is directly proportional to the color intensity of the test sample.

REAGENTS

Materials provided with the kit:

- Antibody-coated microtiter plate, 96 wells.
- Zero Buffer, (12 ml yellow liquid color with transparent cap vial).
- Enzyme Conjugate Reagent, (18 ml red liquid color with transparent cap bottle).
- IgE reference standards, containing 0 (white cap), 10 (yellow cap), 50 (green cap), 100 (blue cap), 400 (purple cap), and 800 (red cap) IU/mL. 0.3 ml in each vial, ready for use.
- TMB Substrate, (12 ml brown cap vial).
- Stop Solution, (12 ml transparent cap vial).
- Wash Buffer Concentrate (50X), (15 mL green cap bottle).

Materials required but not provided:

- Precision pipettes: 10µL ~40µL, 40µL ~200µL and 1.0 mL.
- Disposable pipette tips
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- A microtiter plate reader.
- Graph paper.

SPECIMEN COLLECTION AND PREPARATION

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.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450-630 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-22°C) before use.
2. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, dilute 15 ml of Wash buffer (50x) into 735 mL of distilled water to prepare 750 ml of Washing buffer (1x). Mix well before use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 20 µl of standard, specimens, 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 a complete mixing in this setup.
5. Incubate at room temperature (18-22°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 washing buffer (1X).
8. Strike the wells 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 5 seconds
10. Incubate at room temperature for 30 minutes.
11. Remove the incubation mixture by flicking plate contents into sink.
12. Rinse and flick the microtiter wells 5 times with Washing buffer (1X)
13. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
14. Dispense 100 µl TMB Reagent into each well. Gently mix for 5 seconds.
15. Incubate at room temperature in the dark for 20 minutes.
16. Stop the 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 450-630 nm with a microtiter plate reader.

Important Note: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

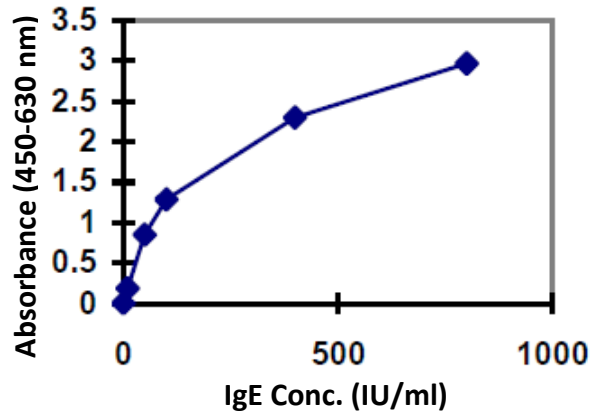
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A450-630) for each set of reference standards, specimens, controls, and patient samples.
2. Constructed a 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 or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample to determine the corresponding concentration of IgE in IU/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450-630 nm shown in the Y-axis against IgE concentrations shown in the X-axis.

IgE Values (IU/ml)	Absorbance (450-630 nm)
0	0.008
10	0.189
50	0.851
100	1.287
400	2.300
800	2.966



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 data and standard curve.

EXPECTED VALUES AND SENSITIVITY

The total IgE level in a normal, allergy-free adult is less than 150 IU/ml of serum. The minimum detectable concentration of IgE by this assay is estimated to be 5.0 IU/ml.

Performance characteristics

1. Accuracy:Comparsion between our Kits and commercial availabel Kits provide the following data

N = 100
Corellation Coeffecient = 0.960
Slope = 1.04
Intercept = 22.02
Mean (Our) = 205.4
Mean (Abbott) = 204.8

2. Precision

1. Intra-Assay:

Concentration	Replication	Mean	S.D	% CV
Level	20	78.60	3.54	4.50
Level I	20	79.07	4.16	5.26
Level II	20	347.28	10.76	3.10

2. Inter-Assay:

Concentration	Replication	Mean	S.D	% CV
Level	20	77.45	6.15	7.94
Level I	20	78.99	4.85	6.14
Level II	20	356.10	14.43	4.05

3. Linearity

A patient serum were serially diluted with 0 IU/mL Standard in a linearity study. The average recovery was 100.3%.

Sample			
Dilution	Expected	Observed	% Recov.
Undiluted	837.4	837.4	
2x	418.7	399.8	95.5
4x	167.5	170.3	102.0
8x	83.7	81.0	96.7
16x	41.9	43.1	103.0
32x	21.0	21.9	104.3
Average Recovery:100.3%			

4. Recovery: Two pooled sera with known IgE were spiked with 300, 500 and 700 IU/mL IgE. The samples were assayed in three separate runs in triplicate. The average recovery was 99.7%.

Samples	Original Con.	Added	Expected	Observed	% Recovery
Sample 1	26.1	300.0	326.1	298.4	91.5
Sample 2	26.1	500.0	526.1	537.1	102.1
Sample 3	26.1	700.0	726.1	737.0	101.5
Sample 4	57.8	300.0	357.8	349.1	97.6
Sample 5	57.8	500.0	557.8	571.2	102.4
Sample 6	57.8	700.0	757.8	780.0	102.9
Average Recovery: 99.7 %					

5. Sensitivity

The sensitivity is defined as the concentration of IgE that corresponds to the absorbance that is two standard deviations greater than the mean absorbance of 20 replicates of the zero calibrator. The minimum detectable concentration of this assay is estimated to be 5.0 IU/mL

6. Cross-reactivity

The following human immunoglobulins were tested for cross reactivity of the assay.

7. Hook Effect

No hook effect was observed in this assay.

Antigens	Concentration	Equivelent IgE
Human IgA	400 mg/dL	< 5.0 IU/mL
Human IgG	400 mg/dL	< 5.0 IU/mL
Human IgM	400 mg/dL	< 5.0 IU/mL

LIMITATIONS OF THE PROCEDURE

1. For professional use only
2. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
3. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot gurantee it will eliminate all the effects of that.

REFERENCES

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry