



Immunoglobulin E (IgE) Product Code: 2525-300

Intended Use: The Quantitative Determination of Immunoglobulin E (IgE) Concentration in Human Serum by a Microplate Enzyme Immunoassay.

SUMMARY AND EXPLANATION OF THE TEST

Allergic reactions, which are becoming more widespread, are usually diagnosed on the basis of medical history and clinical symptoms. In vitro and in vivo testing, however, play a key role in confirming clinical suspicions and tailoring treatment. The measurement of immunoglobulin E (IgE) in serum is widely used in the diagnosis of allergic reactions and parasitic infections. Many allergies are caused by the immunoglobulins of subclass IgE acting as point of contact between the allergen and specialized cells. The IgE molecules (MW 200,000) bind to the surface of the mast cells and basophilic granulocytes. Subsequently the binding of allergen to cell-bound IgE causes these cells to release histamines and other vasoactive substances. The release of histamines in the body results initiates what is commonly known as an allergic reaction.

Before making any therapeutic determination it is important, however, to know whether the allergic reaction is IgE mediated or non-IgE mediated. Measurement of total IgE in serum sample, along with other supporting diagnostic information, can help to make that determination. Measurement of total circulating IgE may also be of value in the early detection of allergy in infants and as a means of predicting future atopic manifestations. Before deciding on any therapy it is important to take into consideration all the relevant clinical information as well as information supplied by specific allergy testing.

IgE levels show a slow increase during childhood, reaching adult levels in the second decade of life. In general, the total IgE levels increase with the allergies a person has and the number of times of exposure to the relevant allergens. Significant elevations may be seen in the sensitized individuals, but also in cases of myeloma, pulmonary aspergillosis, and during the active stages of parasitic infections.

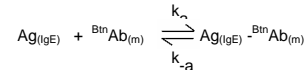
In this method, IgE calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal antibody (specific for IgE) is added and the reactants mixed. Reaction between the IgE antibodies and native IgE forms complex that binds with the streptavidin coated to the well. The excess serum proteins are washed away via a wash step. Another enzyme labeled monoclonal antibody specific to IgE is added to the wells. The enzyme labeled antibody binds to the IgE already immobilized on the well through its binding with the biotinylated monoclonal antibody. Excess enzyme is washed off via a wash step. A color is generated by the addition of a substrate. The intensity of the color generation is directly proportional to the concentration of the IgE in the sample.

PRINCIPLE

Immunoenzymometric sequential assay (TYPE 4):

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-IgE antibody.

Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an antibody-antigen complex. The interaction is illustrated by the following equation:



$B^{in}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)

$Ag_{(IgE)}$ = Native Antigen (Variable Quantity)

$Ag_{(IgE)} - B^{in}Ab_{(m)}$ = Antigen-Antibody complex (Variable Quantity)

k_a = Rate Constant of Association

k_a = Rate Constant of Disassociation

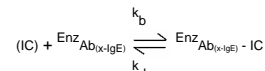
Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

$Ag_{(IgE)} - B^{in}Ab_{(m)} + Streptavidin_{C.W.} \rightarrow Immobilized\ complex\ (IC)$

$Streptavidin_{C.W.}$ = Streptavidin immobilized on well

$Immobilized\ complex\ (IC)$ = Ag-Ab bound to the well

After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce color measurable with the use of a microplate spectrophotometer. The enzyme activity on the well is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.



$Enz_{Ab(x-IgE)}$ = Enzyme labeled Antibody (Excess Quantity)

$Enz_{Ab(x-IgE)} - IC$ = Antigen-Antibodies Complex

k_b = Rate Constant of Association

k_b = Rate Constant of Dissociation

REAGENTS

Provided:

A. Human Serum References -- 1.0 ml/vial - Icons A-F

Six (6) vials of human serum based reference calibrators at concentrations of 0 (A), 5 (B), 25 (C), 50 (D), 150 (E) and 400 (F) IU/ml. Store at 2-8°C. A preservative has been added.

(The Calibrators are standardized against WHO's 2ndIRP 75/502 for IgE).

B. IgE Biotin Reagent -- 13 ml/vial

One (1) vial of biotinylated anti-human IgE mIgG reagent presented in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

C. IgE Enzyme Reagent-- 13 ml/vial - Icon E

One (1) vial of anti-human IgE-HRP incorporated complex in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

D. Streptavidin Plate-- 96 wells - Icon

One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution -- 20ml - Icon

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C.

F. Substrate A --7.0ml/vial - Icon S^A

One (1) bottle containing tetramethylbenzidine (TMB) in acetate buffer. Store at 2-8°C.

G. Substrate B -- 7.0ml/vial - Icon S^B

One (1) bottle containing hydrogen peroxide (H₂O₂) in acetate buffer. Store at 2-8°C.

H. Stop Solution -- 8.0ml/vial - Icon

One (1) bottle containing a strong acid (1N HCl). Store at 2-8°C.

I. Product Insert.

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C.

Note 3: Above reagents are for a single 96-well microplate.

Required But Not Provided:

- Pipette capable of delivering 25 & 50µl volumes with a precision of better than 1.5%.
- Dispenser(s) for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
- Microplate washers or a squeeze bottle (optional).
- Microplate Reader with 450nm and 620nm wavelength absorbance capability.
- Absorbent Paper for blotting the microplate wells.
- Plastic wrap or microplate cover for incubation steps.
- Vacuum aspirator (optional) for wash steps.
- Timer.
- Quality control materials.

PRECAUTIONS

*For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals*

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot for samples. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required.

REAGENT PREPARATION:

1. Wash Buffer

Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature (20-27°C) for up to 60 days.

2. Working Substrate Solution

Pour the contents of vial labeled Solution 'A' into the vial labeled Solution 'B'. Place the yellow cap on the mixed reagent for easy identification. Mix and label accordingly. Store at 2-8 °C.

Note: Do not use the working substrate if it looks blue.

TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).

- Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
- Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.
- Add 0.100 ml (100µl) of the IgE Biotin Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
- Swirl the microplate gently for 20-30 seconds to mix and cover.
- Incubate 30 minutes at room temperature.
- Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
- Add 0.100 ml (100µl) of the IgE Enzyme Reagent labeled antibody to each well. **DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION**
- Cover and incubate 30 minutes at room temperature.
- Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
- Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
- Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time. DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION**
- Incubate at room temperature for fifteen (15) minutes.
- Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds.
- Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of IgE in unknown specimens.

- Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
- Plot the absorbance for each duplicate serum reference versus the corresponding IgE concentration in IU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
- Draw the best-fit curve through the plotted points.
- To determine the concentration of IgE for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in IU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.323) intersects the dose response curve at 142 IU/ml IgE concentration (See Figure 1).

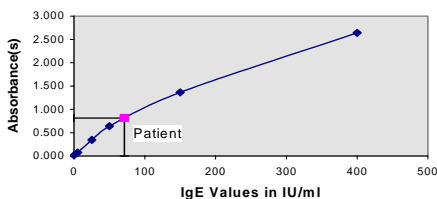
Note: Computer data reduction software designed for IEMA (ELISA) assays may also be used for the data reduction.

EXAMPLE 1

I.D.	Well Position	Absorbance	Mean Absorbance (B)	Concentration
Cal A	A1	0.014	0.015	0
	B1	0.016		
Cal B	C1	0.072	0.073	5
	D1	0.074		
Cal C	E1	0.364	0.345	25
	F1	0.326		
Cal D	G1	0.663	0.639	50
	H1	0.614		
Cal E	A2	1.340	1.364	150
	B2	1.388		
Cal F	C2	2.601	2.641	400
	D2	2.682		
Ctrl 1	E2	2.575	2.562	375.3
	F2	2.549		
Ctrl 2	G2	0.818	0.813	71.2
	H2	0.807		
Patient 1	A3	1.322	1.323	142.0
	B3	1.324		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a standard curve prepared with each assay.

Figure 1



Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

- The absorbance (OD) of calibrator 'A' should be ≤ 0.1 .
- The absorbance (OD) of calibrator 'F' should be ≥ 1.3 .
- Four out of six quality control pools should be within the established ranges.

LIMITATIONS OF PROCEDURE

- It is important that the time of reaction in each well is held constant for reproducible results.
- If more than one (1) plate is used, it is recommended to repeat the dose response curve.
- Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time-deviation during reaction.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
- Use components from the same lot. No intermixing of reagents from different batches.
- Serum IgE concentration is dependent upon a multiplicity of factors: including if the patient is sensitized, how many times the patient has been exposed to a specific allergen etc. Total IgE concentration alone is not sufficient to assess the clinical status. All the clinical findings especially specific allergy testing should be taken into consideration while determining the clinical status of the patient.
- Since all atopic reactions are not IgE mediated, all relevant clinical information should be taken into consideration before making any determination for patients who may be in the normal range.

EXPECTED RANGES OF VALUES

A study of population from different age groups was conducted to evaluate the IgE AccuBind™ ELISA test system. The results are presented in Table 1:

Age (Yrs)	Number (n)	Median	Absolute Range
0-3	31	6.4	ND - 46
3-16	43	25.0	ND - 280
Adult	145	43	0 - 200

Each laboratory is advised to establish its own ranges for normal and abnormal populations. These ranges are always dependent upon locale, population, laboratory, technique and specificity of the method.

PERFORMANCE CHARACTERISTICS

A. Precision

In order to validate the intra-assay precisions of the IgE AccuBind™ ELISA test system, twenty replicates of each of three pooled sera (low medium and high ranges of the dose response curve) were assayed in the same assay. An intra-assay precision of 1.95 to 5.87% was obtained.

	Low	Medium	High
Number	20	20	20
Mean	48.9	160.5	297.6
1 S.D.	2.87	6.47	5.81
% CV	5.87	4.03	1.95

In order to validate the inter-assay precision of the IgE AccuBind™ ELISA test system, one duplicate of each of three pooled sera (low medium and high ranges of the dose response curve) was assayed in 10 assays done over a period of six

months that involved five different sets of reagents and three different technicians. An inter-assay precision of 3.52 to 8.42% was obtained. See Table 3 below.

	Low	Medium	High
Number	10	10	10
Mean	46.3	157	301
1 S.D.	3.9	7.3	10.6
% CV	8.42	4.64	3.52

B. Accuracy

The IgE AccuBind™ ELISA test system was compared with a coated tube radio immunoassay (IRMA) method. Biological specimens with IgE levels in the low, medium and high ranges were used (The values ranged from 0.8 to 3100 IU/ml). The total number of such specimens was 219. The least square regression equation and the correlation coefficient were computed for this IgE AccuBind™ ELISA method in comparison with the predicate method (Table 4):

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
Monobind EIA "X"	179	$x = -12.9 + 1.21(Y)$	0.967
Predicate IRMA "Y"	157		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

C. Linearity

Two patient pools were assayed diluted (in 'A' Calibrator) and undiluted with the IgE AccuBind™ ELISA test system. The observed and expected values are listed below in Table 5:

Sample	Observed	Expected	% Recovery
Pool 1	106.8	-	-
Pool 1/2	50.8	53.4	95.1
Pool 1/4	25.3	26.7	94.8
Pool 1/8	13.4	13.3	100.6
Pool 1/16	6.6	6.7	98.5
Pool 2	395.9	-	-
Pool 2/2	189.5	197.9	95.8
Pool 2/4	106.1	98.9	107.2
Pool 2/8	48.0	49.5	96.9
Pool 2/16	25.8	24.7	104.2

D. Sensitivity

The IgE AccuBind™ ELISA test system has a sensitivity of 1.0 IU/ml. The sensitivity was ascertained by determining the variability of the 0 IU/ml serum calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

E. Specificity

The specificity of the IgE AccuBind™ ELISA test system, to closely related immunoglobulins was evaluated by adding those at twice the physiological concentrations to a serum matrix. No cross-reaction between the antibodies used and the related molecules was detected.

F. Recovery

Two patient pools were spiked with known amounts of IgE and assayed with the IgE AccuBind™ ELISA test system. The observed and expected values are listed below in Table 6.

Sample	Observed (O) IU/ml	Expected (E) IU/ml	% Recovery (O/E)
Pool 1	25.7	-	-
Pool 1+ 25	50.7	50.7	100.0
Pool 1+ 50	74.8	75.7	101.2
Pool 1+ 100	122.7	125.7	97.6
Pool 1+ 200	232.0	225.7	102.7
Pool 2	12.3	-	-
Pool 2+ 25	41.7	37.3	111.2
Pool 2+ 50	62.6	62.3	100.6
Pool 2+ 100	109.4	112.3	97.4
Pool 2+ 200	197.2	212.3	92.8

G. High Dose Effect

Since the assay is sequential in design, high concentrations of IgE do not show the hook effect. Myeloma IgE patient samples with concentrations over 8 million IU/ml demonstrated extremely high levels of absorbance.

REFERENCES

- Plebani M, Bernardi D, Basso D, Faggian, D and Borghesan F, "Measurement of specific immunoglobulin E: intermethod comparison and standardization", *Clin Chem*, **44**, 9 (1998).
- Geha RS, "Human IgE", *J Clinical Immunology*, **74**, 109-120 (1984).
- Barbee RA, et al, "Distribution of IgE in a community population sample: correlation with age, sex and allergen skin reactivity", *J of Clinical Immunology*, **68**, 106-111 (1981).
- Nye L, Marrett TG., Landon J, White RJ, "A detailed investigation of circulating levels of IgE in a normal population", *Clin Allergy*, **1**, 13-24 (1975).
- Mandy FF, Perelmutter L, "Laboratory measurement of total human serum IgE", *Journal Clinical Immunoassay*, **6(2)**, 140-146 (1983).
- Hamilton RG, Adkinson RF, "Clinical laboratory methods and allergic disease", *Lab Management*, **21(12)**, 37-50 (1983).
- Halpern GM, "Markers of human allergic disease", *J Clin Immunoassay*, **6(2)**, 131-139 (1983).
- Homburger HA, Yuningger JW, "Laboratory testing in the diagnosis and management of allergic diseases", *Clin Lab*, **2**, 351-388 (1983).
- National Committee for Clinical Laboratory Standards: Procedures for the collection of blood specimens by venipuncture 3rd Ed, NCCLS Doc H3-A3 (1991).
- Tietz NW, *Clinical Guide to Laboratory Tests*, 3rd Ed, Philadelphia, WB Saunders **358** (1995).

Revision: A Date: 090506

Cat #: 2525-300

For Orders and Inquiries, please contact



Tel: 949-951-2665
Fax: 949-951-3539

Email: info@monobind.com

On the Web: www.monobind.com

Please visit our website to learn more about our other interesting products and services.



EC REP CEpartner4U, 3951 DB; 13.NL
Tel: +31 (0) 6-516.536.26

Instruments & Applications

Monobind's immunoassay products are designed to work in both manual and automated lab environments. AccuBind™ and AccuLite™ are compatible with any open-ended instrumentation, including chemistry analyzers, microplate readers and microplate washers. There may or may not be an application developed for your particular instrument, please visit the instrument section of our website, or contact techsupport@monobind.com

Monobind offers several instruments, including the Impulse 2 Luminometer CLIA Plate Reader designed hand-in-hand with our products and capable of 2-point calibration. Visit our website for more information.