Leinco Technologies, Inc.™

PRODUCT DATA SHEET

359 Consort Drive • St. Louis, Missouri 63011 • 800.538.1145 • Fax: 636.527.5545 • *www.leinco.com*

Human IgE MICRO-ELISA Test Kit

Prod. No.: **T177**
Pkg. Size: **96 Tests**

Description

The MICRO-ELISA IMMUNOGLOBULIN E (IgE) test is a solid phase sandwich-type enzyme immunoassay (ELISA) Diagnostic Kit for the *in vitro* quantitative determination of IgE concentration in human serum.

Summary and Explanation of the Test

The measurement of the IgE class of immunoglobulin is considered extremely valuable in the study of allergic reactions. Significant elevations have been observed in most patients with allergic diseases such as hay fever, atopic eczema, extrinsic asthma and allergic rhinitis.[1-5] An elevated level is also associated with parasitic infections such as hook worm, echinococcus and bilharziasis.[6] Increased levels have also been found in cancer patients.

The determination of IgE levels in hereditarily predisposed children has been used as a tool for predicting future atopic manifestations and potentially as a screening method for children of atopic parents.[5,7-8]

The IgE antibody molecule is composed of two identical heavy chains (ε chains) and two identical light chains (κ or λ) linked by disulfide bridges. The molecule can be cleaved into F_{ab} and F_c fragments by proteolytic enzymes such as pepsin or papain. The F_c region of the IgE (containing the carboxyl-terminal potion of both heavy chains) is constant for all IgE molecules. The F_{ab} region (containing both of the light chains and the other portion of the heavy chains) is variable and contains the antigen (allergen) specific portion of the antibody.[9] Membrane receptors on certain white blood cells bind the Fc portion of the IgE-allergen complex and trigger release of histamine and the appearance of the typical symptoms of allergic reactions such as inflammation, itching and congestion.[8,10]

Levels of IgE are increased in individuals with allergic conditions. This increase in the serum concentration of total IgE is related both to the extent of the allergic reaction and the number of different allergens to which the individual responds.[2-3]

Principle of the Procedure

The MICRO-ELISA IgE test is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes two unique antibodies (a mouse monoclonal and a goat polyclonal) directed against distinct antigenic determinants on the IgE molecule.

Specifically, plastic wells are coated with anti-IgE (mouse monoclonal). With the addition of a test sample or appropriate controls containing IgE, immune complexes are formed between IgE in the sample and the solid phase anti-IgE. Anti-IgE (goat polyclonal) enzyme-labeled with horseradish peroxidase is added to each well. During an incubation period (45 minutes at room temperature), the IgE molecule is sandwiched between the solid phase and enzyme-labeled antibodies. The sample is then decanted and the wells are washed to remove unbound-labeled antibody. An enzyme substrate-chromogen (hydrogen peroxide, H_2O_2, and tetramethylbenzidine, TMB) is added to the well and incubated for 15 minutes at room temperature, resulting in the development of a blue color. The addition of 1.0 N H_2SO_4 stops the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of IgE in the sample.

Reagents

Components in Each 96-Test
Micro-Elisa IgE Diagnostic Kit

1. 96 wells, IgE ANTIBODY COATED **WELLS:** Coated with anti-IgE (mouse monoclonal); contained in a pack with silica gel desiccant.
2. 1 bottle, 27 ml, IgE ENZYME ANTIBODY **CONJUGATE:** anti-IgE (goat polyclonal) labeled with horseradish peroxidase in buffered protein solution; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives; contains FD&C red # 40 as coloring agent.
3. 1 bottle, 12 ml, **SUBSTRATE-CHROMOGEN** Buffered hydrogen peroxide and 3,3',5,5'- tetramethylbenzidine (TMB) solution.
4. 1 bottle, 12 ml, **STOP** SOLUTION 1 N H_2SO_4.
5. 1 vial, 3 ml, **0 IU/ml IgE CALIBRATOR** - Bovine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives.
6. 3 vials, 0.5 ml, IgE **CALIBRATORS:** IgE in bovine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. 100, 250 and 500 IU/ml.
7. 1 bottle, 60 ml, **WASH BUFFER CONCENTRATE (20X):** Buffered detergent solution, contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. Dilute bottle to 1200 ml with deionized water.

Additional Materials Required

Disposable tip precision pipets - 0.01, 0.1 and 0.25 ml.
microtiter plate reader.
Distilled or deionized water.

Storage and Stability

Store unopened kits at 2°-8°C. The following components may be stored at ambient temperature: WELLS,

Products are for research use only. Not for use in diagnostic or therapeutic procedures.

359 Consort Drive • St. Louis, Missouri 63011 • 800.538.1145 • Fax: 636.527.5545 • *www.leinco.com*

SUBSTRATE-CHROMOGEN, WASH BUFFER and STOP SOLUTION. Expiration date printed on the kit indicates limits of stability.

The IgE ANTIBODY COATED WELLS are supplied in a resealable bag containing a desiccant and must be stored with the bag sealed to protect from moisture. Wells can be stored at 2°-30°C.

Chemical or Physical Indications of Instability

Alterations in the physical appearance of reagents, or results consistently outside the acceptable limits for control sera, may be due to reagent contamination or deterioration.

Instruments

Performance of the MICRO-ELISA IgE test requires use of a precision microtiter plate reader a wavelength of 450 ± 20 nm:

Specimen Collection and Preparation

Serum samples are used in the MICRO-ELISA IgE Diagnostic Kit procedure. No special preparation of the patient is necessary; fasting is not required. Repeated freezing and thawing of specimens should be avoided. No additives or preservatives are necessary.

STORAGE: Specimens may be stored in a tightly sealed tube at 2°-8°C for three days. If the serum is not assayed within 3 days, store frozen (-20°C) in a tightly sealed tube for up to 3 weeks. Specimens should be allowed to come to room temperature and should be mixed thoroughly by gentle inversion before assaying.

Do not use grossly lipemic specimens. Moderately lipemic, hemolyzed and icteric specimens should not interfere with the assay.

Micro-ELISA IgE Procedure

Reagent Preparation

Dilute bottle of WASH BUFFER CONCENTRATE (20X) solution to 1200 ml with deionized water. Diluted wash solution will be stable until the expiration date stamped on the kit.

Preliminary Comments and Precautions

Patient sample may contain pathogens: treat all samples as potentially infectious.

Reagents contain thimerosal; avoid contact with skin.

Avoid contact with SUBSTRATE-CHROMOGEN (tetramethyl-benzidine) solution. It is harmful if inhaled or absorbed through skin (may cause irritation).

CAUTION: Source material used to prepare Calibrators was derived from human material. The material was tested using FDA-approved methods and found non-reactive for Hepatitis B Surface Antigen (HBsAg) by ELISA and non-reactive for HIV by ELISA. No known test method can offer total assurance that infectious agents are absent. HANDLE THESE REAGENTS AS IF THEY ARE POTENTIALLY INFECTIOUS. Information on handling human serum is provided in the CDC/NIH manual "Bio-safety in Microbiological and Biomedical Laboratories" (1984).

Procedural Notes

1. All test kit components used in an assay must be of the same master lot number. Materials should not be used after the expiration date shown on the package label. Components and test specimens should be at room temperature (18°-30°C) before testing begins.
2. All calibrators, controls, and samples should be tested in duplicate simultaneously. The test samples and controls must be well mixed before use.
3. A separate disposable tip should be used for each sample to avoid cross-contamination. All pipetting steps should be performed with the utmost care and accuracy. Avoid contaminating the reagent pipette tip with the serum sample.
4. The duration of the incubation times must be the same for all wells within a run.
5. Run size should be limited to the number of samples that can be added to antibody coated wells within 5 minutes.
6. Samples should be pipetted to the bottom of the antibody-coated wells.
7. If microtiter reader is not capable of reading absorbances greater than 2.0, the color should be read after a shorter incubation time with the SUBSTRATE/CHROMOGEN, i.e., 10 minutes.

Test Procedure

1. Place sufficient COATED WELLS in a holder to run 0.0, 100, 250 and 500 IU/ml IgE CALIBRATORS, Quality Control Sera and patient samples in duplicate. Limit run size to the number of samples that can be pipetted in 5 minutes.
2. Pipet 10 µl of the CALIBRATORS, Controls or Patient Sample to the corresponding COATED WELL.
3. Pipet or dispense 250 µl of the ENZYME ANTIBODY CONJUGATE solution to all the wells and mix gently.
4. Incubate at room temperature (18°-30°C) for 45 minutes ± 5 minutes.
5. Decant or aspirate and discard liquid contents of all wells. SLAP the inverted wells on a clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
6. Fill each well with diluted WASH BUFFER. Fill the wells to overflowing, you cannot cause any carryover between the wells. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.

Leinco Technologies, Inc.

PRODUCT DATA SHEET

359 Consort Drive • St. Louis, Missouri 63011 • 800.538.1145 • Fax: 636.527.5545 • www.leinco.com

WARNING: WASHING THE WELLS IS OF CRITICAL IMPORTANCE. Fill the wells to overflowing, you CANNOT cause any carryover between wells. You CANNOT over wash the wells. Completely decant or aspirate all of the liquid out of the wells. SLAP the inverted wells on a FRESH clean piece of absorbent paper AFTER EACH WASH. YOU CANNOT SLAP TOO HARD, REMOVE ALL OF THE LIQUID FROM THE WELLS.

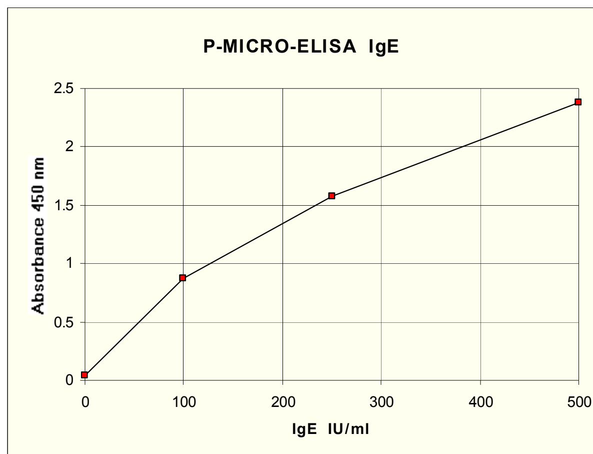
7. Repeat step 6 three more times (for a total of 4 washes).
8. Fill each well with deionized water. Fill the wells to overflowing. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
9. Pipet or dispense 100 µl (0.1 ml) of SUBSTRATE-CHROMOGEN solution into each well.
10. Mix thoroughly and incubate 15 minutes at room temperature (18°-30°C).
11. Pipet or dispense 100 µl (0.1 ml) of 1 N H_2SO_4 into each well and mix thoroughly.
12. Read the absorbance of each well at 450 ± 20 nm against water.

Calculation of Results

1. Calculate the mean value for each duplicate sample absorbance. Values for duplicate absorbances should be within 10% (or 0.02 absorbance units for absorbances less than 0.2).
2. Construct the standard curve by plotting the mean absorbance obtained for each IgE CALIBRATOR on the vertical (Y) axis versus the corresponding IgE concentration on the horizontal (X) axis, using rectilinear graph paper.
3. Connect the points with straight-line segments.
4. Using the mean absorbance for each sample, read the corresponding IgE concentration in IU/ml from the curve. Multiply the value by the dilution factor if required.

EXAMPLE DATA
DO NOT USE IN PLACE OF CUREVE
DETERMINED ATHE THE TIME OF ASSAY

Specimen I.D.	A_{450}	Mean A_{450}	IgE(IU/ml)
CALIBRATOR	0 IU/ml	0.040, 0.036	0.038
CALIBRATOR	100 IU/ml	0.868, 0.886	0.877
CALIBRATOR	250 IU/ml	1.626, 1.533	1.580
CALIBRATOR	500 IU/ml	2.355, 2.406	2.381
SAMPLES			
# 1 (UNKNOWN #l)	0.795, 0.838	0.817	93
# 2 (UNKNOWN #2)	0.732, 0.731	0.732	83
# 3 (UNKNOWN #3)	1.928, 1.944	1.936	361



The range of this assay is 0 - 500 IU/ml. For specimen with IgE concentrations beyond the standard curve (500 IU/ml), repeat the test by diluting the specimen with the 0 IU/ml CALIBRATOR. To obtain the final concentration, multiply the concentration of the diluted sample by the dilution factor.

Quality Control

Good laboratory practice requires that quality control specimen be run with each calibration curve to check the assay performance. Controls containing azide can not be used. Two controls with normal and elevated values should be used. Pooled human serum or commercially available control sera without azide are suitable. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

Read the absorbance of the test solutions against a water blank. If the absorbance of the 0 IU/ml CALIBRATOR exceeds 0.100 it is an indication of careless washing and the assay must be repeated.

Standardization

The MICRO-ELISA IgE CALIBRATORS have been standardized against the World Health Organization International Reference Preparation (2nd IR 75/502).

Limitations of the Procedure

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.

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). These samples should not be assayed with the MICRO-ELISA IgE test as erroneous results may be

Products are for research use only. Not for use in diagnostic or therapeutic procedures.

359 Consort Drive • St. Louis, Missouri 63011 • 800.538.1145 • Fax: 636.527.5545 • *www.leinco.com*

obtained. These conditions should be ruled out prior to clinical evaluation of test results.

The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbances. The use of tap water for washing could result in a higher background absorbance.

FINAL REACTION STABILITY: The spectrophotometric measurement should be made within 30 minutes after the addition of the H_2SO_4 solution.

Samples with elevated levels of IgE (up to 15,000 IU IgE/ml) will always assay as >500 IU IgE/ml when tested, and will not result in a "high dose hook effect". When it is necessary to measure levels of IgE greater than the 500 IU IgE/ml CALIBRATOR, the sample should be diluted with the 0 IU/ml CALIBRATOR and re-assayed.

Expected Values

IgE values were measured in 282 serum samples from apparently normal individuals and the following results were obtained:

	N	Geometric Mean	IgE (IU/ml) Observed Value
ADULT FEMALE	171	35	1 – 719
ADULT MALE	111	40	1 - 730

% of Sample population	IgE values were below:
90%	less than 220 IU/ml
75%	less than 80 IU/ml
50%	less than 35 IU/ml

These values are consistent with those reported in the literature. Values up to 150 IU/ml are reported to be consistent with non-allergic individuals.[7] Elevated IgE levels are strongly indicative of atopic disease.[1-5] Serum IgE concentrations in children without atopic incident are approximately 15% of the adult values.[7]

IgE values for normal pediatric population:

Age	Range
4-11 months	1-19 IU/ml
1 year	2-38 IU/ml
2 years	2-30 IU/ml
3-6 years	5-55 IU/ml
7-9 years	7-123 IU/ml
10-14 years	12-132 IU/ml
15-19 years	11-93 IU/ml
Adult (non-allergic)	<150 IU/ml

These values are consistent with those reported in the literature.
It is recommended that each laboratory determine its own normal range.

Performance Characteristics of the Test

Assay Specificity

The antibodies used in the MICRO-ELISA IgE kit exhibit no significant cross-reactivity to other human immunoglobulin (IgG, IgM, IgA or IgD) at physiological concentrations.

Assay Sensitivity

The sensitivity of this assay is defined as the smallest single value that can be distinguished from the zero calibrator. This value was calculated from the mean + two standard deviations for twenty-one replicates at the zero concentration. The calculated sensitivity is <1.0 IU/ml.

Assay Reproducibility

Intra-assay reproducibility was determined by measurement of 21 replicates of three serum pools in a single run.

	Mean IgE (IU/ml)	SD	%CV
Serum A	75.51	4.03	5.3
Serum B	125.59	7.02	5.6
Serum C	372.40	28.94	7.8

The interassay reproducibility was determined by duplicate measurement of three serum pools in nine separate runs.

	Mean IgE (IU/ml)	SD	% CV
Serum A	70.39	4.41	6.3
Serum B	122.69	11.12	9.0
Serum C	368.62	26.98	7.3

Assay Linearity

A study was performed diluting a serum sample containing an elevated level of IgE with the 0 IU/ml CALIBRATOR to determine the linearity of the MICRO-ELISA IgE.

	IgE IU/ml		
Dilution Factor	Expected Value	Observed Value	% of Expected Value
undiluted	-	452	-
1:2	226	214	95 %
1:4	113	116	103 %
1:8	57	61	107 %

Assay Recovery

Three aliquots of human sera with a IgE concentrations of 8.0 IU/ml were spiked with 10, 25, 50, and 100 µl of a serum with an IgE concentration of 448 IU IgE/ml. The samples were assayed in duplicate.

	IgE IU/ml		
Added Serum 448 IU/ml	Expected Value	Measured Value	% Recovery
0 µl	8.0		
10 µl	16.6	16.0	96 %
25 µl	28.9	27.8	96 %
50 µl	48.0	44.4	93 %
100 µl	81.3	81.8	101 %

Comparison to Other IgE Tests

359 Consort Drive • St. Louis, Missouri 63011 • 800.538.1145 • Fax: 636.527.5545 • *www.leinco.com*

Correlation studies on a random group of 223 serum samples with a range of values from 1 - 690 IU/ml, were performed using the quantitative results from the MICRO-ELISA IgE Test and another ELISA IgE test. The correlation coefficient of the test results was 0.98

		Slope	Y-Intercept	Correlation Coefficient
ELISA	n= 223	0.984	-9.86	0.98

References

1. Halpern, G. M. (1983) *J. Clin. Immunoassay* **6**(2):131
2. Hamiton, R. G. *et al.* (1983) *Lab. Management* **21**(12):37
3. Homberger, H. A. *et al.* (1983) *Clin. Lab. Ann.* **2**:351
4. Mandy, F. F. *et al.* (1983) *J. Clin. Immunoassay* **6**(2):140
5. Norman, P. S. 1985) *Hosp. Prac.* **10**(8):41
6. Capron, A. *et al.* (1975) *IRCS Med. Sci.* **3:** 477
7. Barbee, R. A. *et al.* (1981) *J. Allergy Clin. Immunol.* **68:**106
8. Gordon, R. R. *et al.* (1982) *Lancet* **I:** 72
9. Dorrington, K. J. *et al.* (1978) *Immunol. Rev.* **41**:3
10. Geha, R. S. *et al.* (1984) *Scand. J. Allergy Clin. Immunol.* **74**:109,
11. Davis, J. S: (1979) in *Rheumatology & Immunology* New York, Grune & Stratton, Inc. p 77
12. Highton, J. *et al.* (1984) *J. Immunol. Meth.* **68**:185
13. Masseyeff, R. *et al.* (1975) *J. Immuno. Meth.* **8**:223
14. Santen, R. J. *et al.* (1973) *J. Clin. Invest.* **52**:2616
15. Odell, W. D. *et al.* (1967) *J. Clin. Invest.* **46**:248
16. Primus, F. J. *et al.* (1988) *Clin. Chem.* **34** 261
17. Hansen, H. J. *et al.* (1989) *Clin. Chem.* **35**:146
18. Schroff, R. J. *et. al.* (1985) *Cancer Res.* **42** :879

Products are for research use only. Not for use in diagnostic or therapeutic procedures.