

Total IgE

Immunoglobulin E (ELISA)

REF: DS167751



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Intended use

The DiaSino Total IgE assay is an enzyme-linked immunosorbent assay (ELISA) for the in vitro quantitative determination of Immunoglobulin E (Total IgE) in human serum.

Summary¹⁻⁹

Indications: allergic diseases, helminthiasis, eczematous or non eczematous dermatitis, IgE myeloma, etc. Diagnosis of allergic reactions and atopic diseases: in addition to skin and provocation tests and detection of specific IgE, the diagnosis of allergic diseases also includes detection of total IgE level. However, allergic reactions were not always accompanied by an increase in total IgE levels (adults > 100 IU/ml). On the contrary, low levels of IgE (adult < 25 IU/ml) can not rule out allergic reaction. Through long-term desensitization treatment and keeping away from allergens, the total IgE titer usually decreases. By measuring the total IgE level, allergic asthma and endogenous asthma, allergic rhinitis and vasomotor rhinitis, as well as infant atopic dermatitis and seborrheic dermatitis can be distinguished. High concentrations of IgE (thousands of IU/ml) were found in patients with atopic dermatitis. Other allergic (and high IgE) diseases include acute recurrent or chronic urticaria, recurrent Quincke edema (angioneurotic edema), gastrointestinal intolerance, and rashes of unknown origin. Detection of total IgE can also be used in the differential diagnosis of pulmonary eosinophilic infiltration, allergic aspergillosis, exogenous allergic alveolitis (farmer's lung and pigeon's lung) and church strau β syndrome. IgE in other diseases: non allergic diseases with high IgE levels include various forms of helminthiasis, such as toxocariasis, ascariasis, schistosomiasis, hookworm disease, leishmaniasis and trichonematodiasis. However, no increase of IgE level was found in taeniasis and enterobiasis. For most cases, after effective treatment, IgE level can be reduced to normal range. High concentrations of IgE can be detected in the following diseases: eczematous or non eczematous dermatitis, IgE myeloma, Acute systemic lupus erythematosus (SLE), Graft versus host response, T cell defect (Wiskott Aldrich syndrome), second or third degree burns, Otorhinolaryngological tumors, Liver disease (especially related to alcohol abuse), Late stage of AIDS (CD4 + T cells decreased significantly) IgE deficiency may occur in the following diseases: x-chromosome-related hypogammaglobulinemia, Severe combined immunodeficiency (SCID), and Pulmonary fibrosis disease.

Test principle

Sandwich principle. Total duration of assay: **80 minutes**.

- Sample, Anti-IgE coated microwells and enzyme labeled Anti-IgE are combined.
- During the incubation, IgE presents in the sample is allowed to react simultaneously with the two antibodies, resulting in the IgE molecules being sandwiched between the solid phase and enzyme-linked antibodies.
- After washing, a complex is generated between the solid phase, the IgE within the sample and enzyme-linked antibodies by immunological reactions.
- Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
- The absorbance is proportional to the amount of IgE in the sample.

Reagents

Materials provided

- Coated Microplate - symbol** **IgE PLATE** 8 x 12 strips, 96 wells, pre-coated with mouse monoclonal Anti-IgE.
- Calibrators - symbol** **IgE CAL A-F** 6 vials, 1 mL each, ready to use; Concentrations: 0(A), 10(B), 50(C), 200(D), 400(E) and 800(F) IU/mL.
- Enzyme Conjugate - symbol** **IgE CONJ** 1 vial, 11 mL of HRP (horseradish peroxidase) labeled mouse monoclonal Anti-IgE in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.1% ProClin300 preservative.
- Substrate - symbol** **SUBSTRATE** 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- Stop Solution - symbol** **STOP** 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- Wash Solution Concentrate - symbol** **WASH 40X** 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- IFU - 1 copy.**
- Plate Lid: 1 piece.**

Materials required (but not provided)

- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50 μ L with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings

- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.

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- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Storage and stability

- Store at 2-8°C.
- Seal and return unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation

- Human serum is recommended for this assay.
- Cap and store the samples at 18-25 °C for no more than 8 hours. Stable for 7 days at 2-8°C, and 1 month at -20°C. Freeze only once.
- Do not use heat-inactivated samples.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
- Avoid grossly hemolytic, lipemic or turbid samples.

Quality control

Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. The controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

Wash solution (40X dilution)

Add deionized water to the 40X concentrated Wash Solution Concentrate. Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Test procedure

Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement. Mix all reagents through gently inverting prior to use.

- Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.
- Add **25 μ L of calibrators or samples** to each well.
- Add **100 μ L of enzyme conjugate** to each well.
- Shake the **microplate** gently for **30 seconds** to mix.
- Cover the plate with a plate lid and incubate at **37 °C for 60 minutes**.
- Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- Add **350 μ L of wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of **5 washes**. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
- Add **100 μ L of substrate** to each well.
- Cover and incubate at **ambient temperature (18-25°C)** in the dark for reaction for **20 minutes**. Do not shake the plate after substrate addition.
- Add **50 μ L of stop solution** to each well.
- Shake for **15-20 seconds** to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
- Read the absorbance of each well at **450 nm** (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within **30 minutes** of adding the stop solution.

Calculation

- Record the absorbance obtained from the printout of the microplate reader.
- Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
- Plot the common logarithm of absorbance against concentration in IU/ml for each calibrator.

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- Draw the best-fit curve through the plotted points on linear graph paper. Point-to-Point method is suggested to generate a calibration curve.

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Sample	Value (IU/mL)	Absorbance
Calibrator A	0	0.015
Calibrator B	10	0.121
Calibrator C	50	0.456
Calibrator D	200	1.211
Calibrator E	400	1.936
Calibrator F	800	2.875
Control 1	110	0.933
Control 2	376	1.892
Sample	131	0.982

Limitations - interference

- The assay is unaffected by icterus (bilirubin < 600 µmol/L or < 35 mg/dL), hemolysis (Hb < 0.559 mmol/L or < 0.9 g/dL), lipemia (Intralipid < 1200 mg/dL).
- Criterion: Recovery within ± 10 % of initial value.
- Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
- There is no high-dose hook effect at IgE concentrations up to 10000 IU/mL. In vitro tests were performed on 26 commonly used pharmaceuticals.
- The presence of autoantibodies may induce high molecular weight complexes (macro-IgE) which may cause unexpected high values of IgE.
- Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human Anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
- Genetic variations or degradation of intact IgE into subunits may affect the binding characteristics of the antibodies and influence the final result. Such samples normally exhibit different results among various assay systems due to the reactivity of the antibodies involved.
- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range

1.2-800 IU/mL (defined by the lower detection limit and the maximum of the master curve). The functional sensitivity is 1.2 IU/mL. Values below the detection limit are reported as < 1.2 IU/mL. Values above the measuring range are reported as > 800 IU/mL (or up to 500 IU/mL for 10-fold diluted samples).

Lower detection limit

1.2 IU/mL

The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Expected values

Age	Normal range, IU/mL
Newborns	< 1.4
1-6 months	< 7.5
7-12 months	< 13
1-5 years old	< 58
6-9 years old	< 167
10-15 years old	< 202
> 16 years old	< 97

Specific performance data

Representative performance data are given below. Results obtained in individual laboratories may differ.

Intra-assay precision	:	5.8%
Inter-assay precision	:	6.5%
Inter-lot precision	:	6.7-7.5%
Analytical sensitivity	:	7.5 IU/mL
Recovery	:	87-97%
Linearity	:	95-125%
Cross-reactivity	:	No cross-reactivity to Immunoglobulin G
Interferences	:	No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL
Clinical specificity	:	100%
Clinical sensitivity	:	100%

Method comparison

A comparison of the DiaSino IgE assay (y) with the Roche Elecsys IgE (x) using clinical samples gave the following correlations:

Number of samples measured: 188

Linear regression

$$y = 1.011X - 0.879$$











$$r = 0.9789$$

The sample concentrations were between approx. 1.5 and 500 IU/mL.

References

- Klink M, Cline MG, Halonen M, Burrows B. Problems in defining normal limits for serum IgE. J Allergy Clin Immunol; 85: 440-444 (1990)
- Kerkhof M, Droste JHJ, de Monchy JGR, Schouten JP, Rijcken B. Distribution of total serum IgE and specific IgE to common aeroallergens by sex and age, and their relationship to each other in a random sample of the Dutch general population aged 20-70 years. Allergy; 51: 770-776 (1996)
- Kerstjens HAM, Schouten JP, Brand PLP, Schoonbrood DFME, Sterk PJ, Postma DS. Importance of total serum IgE for improvement in airways hyperresponsiveness with inhaled corticosteroids in asthma and chronic obstructive pulmonary disease. Am J Respir Crit Care Med; 151: 360-368 (1995)
- Omenaas E, Bakke P, Elsayed S, Hanoa R, Gulsvik A. Total and specific serum IgE levels in adults: relationship to sex, age and environmental factors. Clin Exp Allergy; 24: 530-539 (1994)
- Secord EA, Kleiner GI, Auci DI, Smith-Norowitz T, Chice S, Finkielstein A, Nowakowski M, Fikrig S, Durkin H.G. IgE against HIV proteins in clinically healthy children with HIV disease. J Allergy Clin Immunol; 98:979-984 (1996)
- Barbee RA, Halonen M, Kaltenborn W, Lebowitz M, Burrows B. A longitudinal study of serum IgE in a community cohort: Correlations with age, sex, smoking, and atopic status. J Allergy Clin Immunol; 79: 919-927 (1987)
- Elkayam O, Tamir R, Pick AI, Wyszynski A. Serum IgE concentrations, disease activity, and atopic disorders in systemic lupus erythematosus. Allergy; 50: 94-96 (1995)
- Yates VM, Kerr REI, Frier K, Cobb SJ, MacKie RM. Early diagnosis of infantile seborrheic dermatitis and atopic dermatitis – total and specific IgE levels. British Journal of Dermatology; 108: 639-645 (1982)
- Vidal C, Quintela AG, Millán I, Gude F, Cuervas-Mons V. Serum IgE levels in liver cirrhosis. Contrasting results in alcoholic and non-alcoholic patients. Clin Exp Allergy; 24: 540-548 (1994)

Symbols

 In vitro diagnostic medical device	 Temperature limit	 Consult instructions for use	 Catalog number
 Batch code	 Date of manufacture	 Use-by date	 Contains sufficient for <n> tests
 Manufacturer	 Do not use if package is damaged and consult instructions for use		



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