Total IgE
Immunoglobulin E (ELISA)

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

Summary
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 diseasesalso 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 SUSTRATE** 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 50µl 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.
• 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 µL of calibrators or samples to each well.
• Add 100 µL 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 µL 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 µL 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 substate addition.
• Add 50 µL 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 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

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