

Total IgE ELISA

(Immunoglobulin E)

Enzyme immunoassay (microtiter strips) for the quantitative determination of immunoglobulin E (IgE) in human serum and plasma.

REF **RE59061**

 **12x8**

   **2-8 °C**

EU: **IVD**  U.S.: *For research use only.*
Not for use in diagnostic procedures.



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1. INTENDED USE

The Total IgE ELISA Test Kit has been designed for the detection and the quantitative determination of total IgE antibodies in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of IBL-Hamburg.

This assay is intended for in-vitro diagnostic use only.

Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

2. INTRODUCTION

The existence of IgE in man as a unique class of immunoglobulins which are important in the mediation of the allergic response has been known for over twenty years. The mechanism of action involves an initial antigenic stimulation of immunocompetent B lymphocytes by a specific antigen, a process which induces the lymphocyte to respond by producing specific antibody of several classes.

One class, reagenic or IgE antibody, becomes partially bound via its Fc portion to receptors on the surface of mast cells and basophilic leukocytes. Upon further stimulation by specific allergens, these cell-bound IgE molecules bind via their Fab portion to the allergen. This combination triggers the mast cells and basophilic leukocytes to release various vasoactive amines into the blood and the surrounding tissue. These substances cause smooth muscle constriction and lead ultimately to allergic conditions such as wheal and flare reactions, hives, dermatitis, rhinitis, hay fever, asthma and anaphylactic shock.

IgE determinations are most valuable in the diagnostic assessment of patients with established or suspected allergic disease. In normal subjects, IgE values are related to age, with normal values peaking around 10 - 14 years. Infants and children with family history of atopic allergy are at increased risk of developing disease and constitute a prime population for screening. Studies have shown that conditions such as asthma, rhinitis, eczema, urticaria, dermatitis and some parasitic infections lead to increased IgE levels. Asthma, hay fever and atopic eczema patients may produce levels 3 - 10 times those of normal patients.

3. PRINCIPLE OF THE TEST

The Total IgE ELISA is based on the principle of the enzyme immunoassay (EIA). A monoclonal mouse-anti-human IgE antibody is bound on the surface of the microtiter strips. Undiluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate together with anti-human-IgE-peroxidase conjugate. A sandwich complex between the serum IgE and the two antibodies develops. After a 30 minutes' incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then the substrate (TMB) solution is pipetted and incubated for 15 minutes, inducing the development of a blue dye in the wells. The colour development is terminated by the addition of a stop solution, which changes the colour from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgE antibodies is directly proportional to the intensity of the colour.

4. LIMITATION, PRECAUTIONS AND GENERAL COMMENTS

Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.

- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.

- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- No reagents from different kit lots have to be used, and they should not be mixed with one another.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.
- In case of any severe damage of the test kit or components, IBL have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be use for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.
- The disposal of the kit must be made according to the national official regulations. Special information for this product are given in the Material Safety Data Sheets. Safety Data Sheets for this product are available on the homepage of IBL or upon request directly from IBL-Hamburg.

5. REAGENTS PROVIDED

Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18 - 25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2 - 8°C. The opened kit should be used within three months.

Quantity	Symbol	Component
1 x 12 x 8	MTP	Microtiter Plate 12 x 8 well strips coated with Monoclonal anti-IgE. Ready to use!
1 x 1 mL 5 x 0.2 mL	CAL	Standards A - F 0.2 ml each (Standard A: 1 ml), human serum diluted with PBS. Calibrated against the 2 nd International Standard 75/502. Addition of 0.1% sodium azide, containing the following concentrations in [U/mL]: 0; 5; 25; 100; 250; 1000. Ready to use!
1 x 20 mL	ENZCONJ	Enzyme Conjugate goat anti-human-IgE-HRP, in protein-containing buffer solution. Ready-to-use.
1 x 60 mL	WASHBUF CONC	Wash Buffer, 10 x concentrate PBS + Tween 20.
1 x 12 mL	TMB SUBS	Substrate Solution TMB (tetramethylbenzidin). Ready-to-use!
1 x 12 mL	TMB STOP	Stop Solution 0.5 M sulphuric acid. Ready to use!
2 x	FOIL	Adhesive Foil to cover the microtiter strips during the incubation.
1 x		Plastic Bag Resealable, for the dry storage of non-used strips.

6. MATERIALS REQUIRED BUT NOT PROVIDED

- 10 µL-, 200 µL- and 500 µL micro- and multichannel pipets
- Microtiter Plate Reader (450 nm)
- Microtiter Plate Washer

- Reagent tubes for the serum dilution
- Bidistilled water

7. STORAGE AND STABILITY OF THE KIT

The Total IgE ELISA kit is shipped at ambient temperature and should be stored at 2 - 8 °C.

Once the foilbag of the coated microtiter strips has been broken, care should be taken to close it tightly again. The immunoreactivity of the coated microtiter strips is stable up to 3 months in the broken, but tightly closed bag when stored at 2 - 8 °C.

8. SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (4 - 8 °C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples and the standards have to be used undiluted.

9. PREPARATION OF REAGENTS

Allow all reagents and required number of strips to reach room temperature prior to use.

9.1 Wash Buffer

Dilute the Wash Buffer Concentrate with distilled water 1 to 10 (1 + 9) (e. g. 30 ml concentrate + 270 ml distilled water). If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 °C for 15 minutes. The diluted Wash Buffer is stable for 2 months at room temperature.

10. ASSAY PROCEDURE

10.1. General Remarks

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- Standards and samples should be assayed in duplicates.
- A standard curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 4-8 °C.

10.2. Assay Steps

1. Prepare a sufficient amount of microtiter wells for the standards and samples in duplicate as well as for a substrate blank.
2. Pipet **10 µL** each of the **undiluted** samples and the **ready-to-use** standards together with **200 µL** of **conjugate** into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the enclosed foil and incubate at **room temperature for 30 minutes**.
4. Empty the wells of the plate (dump or aspirate) and add **300 µL of diluted washing solution**. This procedure is **repeated totally three times**. Residuals of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
5. Pipet **100 µL** each of the **ready-to-use substrate** into the wells. This time also the substrate blank is pipetted.
6. Cover plate with the enclosed foil and incubate at room temperature for 15 minutes in the dark (e.g. drawer).
7. To terminate the substrate reaction, pipet **100 µL** each of the **ready-to-use stop solution** into the wells. Pipet also the substrate blank.
8. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at **450 nm** (optionally reference wavelength of 620 nm). **The color is stable for at least 60 minutes.**

11. CALCULATION OF RESULTS

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%.

Example

	OD Value	corrected OD	Mean OD Value
Substrate Blank	0,015		
Standard 0 IU/mL	0.030 / 0.036	0.015 / 0.021	0.018
Standard 5 IU/mL	0.070 / 0.054	0.055 / 0.039	0.047
Standard 25 IU/mL	0.162 / 0.148	0.147 / 0.133	0.140
Standard 100 IU/mL	0.646 / 0.604	0.631 / 0.589	0.610
Standard 250 IU/mL	0.974 / 1.014	0.959 / 0.999	0.979
Standard 1000 IU/mL	2.007 / 1.867	1.992 / 1.852	1.922

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently **no reference values** which have to be found in other laboratories in the same way.

The ready to use calibrators of the Total IgE ELISA are defined and expressed in International Units (IU). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible.

On a semilogarithmic graph paper the concentration of the standards (abscissa, logarithmic) are plotted against their corresponding optical density (ordinate, linear). Alternatively, the optical density of each standard and sample can be related to the optical density of the zero standard, expressed as the ratio OD/OD_{max}, and then plotted on the ordinate.

Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.

The concentration of the samples can be read directly from this standard curve by using their average optical density. For the accuracy of the results obtained see chapter 7 (Assay Characteristics). Any sample reading greater than the highest standard should be diluted appropriately with zero standard and reassayed. The result has to be multiplied by the corresponding dilution factor.

Do not use the above calibration curve. In the laboratory the standard curve should be established in each assay run.

12. ASSAY CHARACTERISTICS

Total Immunoglobulin ELISA	
Intra-Assay-Precision	5.1 %
Inter-Assay-Precision	4.1 %
Inter-Lot-Precision	1.3 – 5.9 %
Analytical Sensitivity	0.8 IU/mL
Recovery	87 – 97 %
Linearity	95 – 126 %
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 %

13. QUALITY CONTROL

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test. It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or IBL-Hamburg directly.

14 LIMITATIONS OF USE

Any improper handling of samples or modification of this test might influence the results. Interferences caused by improper sample handling are explained in the chapters 'Specimen Collection and Handling'. Azide and thimerosal at concentrations higher than 0.1 % interfere in this assay. Therefore control sera or samples containing higher concentrations of the above mentioned components may give false results.

15. LEGAL ASPECTS

15.1 Complaints

Complaints will only be accepted in written format (preferably on the manufacturer's complaint form) and only if all details of the test kit, as well as the test results, are included. A copy of the complaint form is available from IBL-Hamburg upon request.

15.2 Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 13. Any laboratory result is only a part of the total clinical picture of a patient. Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutical consequences be derived. The test result itself should never be the sole determinant for deriving any therapeutical consequences.

15.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement. Claims submitted due to customer misinterpretation of laboratory results subject to point 15.2 are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

16. REFERENCES

1. Ishizaka K, Ishizaka T, Hornbrook MM. Physicochemical Properties of Human Reaginic Antibody IV. Presence of a Unique Immunoglobulin as a Carrier of Reaginic Activity. J. Imm., 97: 75 (1966).
2. Hamilton R. Radioimmunoassay in the Assesment of Allergic Disease. Ligand Quarterly, 2: 13 (1979).
3. Johansson S, Bennich H, Berg T. The Clinical Significance of IgE. Progress in Clin. Immunology, 1: (1972).
4. Kjellmann M. Immunoglobulin E and Atopic Allergy in Childhood. Linköping University Medical Dissertations, Nr. 36, (1976).
5. Wittig H, Bellott J, Fillippi I, Royal G. Age-Related Serum IgE Levels in Health Subjects and in Patients with Allergic Disease. J. Allergy Clin. Immunol., 66: 305 (1980).
6. Gleich G, Averbeck A, Swedlund H. Measurement of IgE in Normal and Allergic Serum by Radioimmunoassay. J. Lab. and Clin. Med., 77: 690 (1971).

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλισμένο
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p style="text-align: center;"> Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ. </p>	

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LIABILITY: Complaints will be accepted in each mode –written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer