Product information

Useŕs Manual

Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
fax 02-92157285
info@listarfish.it
www.listarfish.it

Total IgE ELISA

Enzyme immunoassay for the detection and quantitative determination Total Immunoglobulin E (IgE) in serum and plasma

CE IVD

REF **DEIGE02**

Σ **96 wells**

Version 7a
JS/DL
Updated 161125

CONTENTS / INHALTSVERZEICHNIS

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 Demeditec. 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. GENERAL INFORMATION

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, reaginic 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 leucocytes 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. LIMITATIONS, 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.

5. REAGENTS PROVIDED

Symbol	Components	Volume / Qty.
SORB MT	Monoclonal anti-IgE coated microtiter strips	12
CAL A – F	Calibrators (Standards) (0, 5, 25, 100, 250, 1000 IU/mL)	1x 1 mL ; 5x 200 µL
ENZ CONJ	Enzyme Conjugate (goat anti-IgE-HRP)	22 mL
SUB TMB	Substrate	12 mL
STOP SOLN	Stop Solution	12 mL
WASH SOLN 10x	Washing Buffer (10×)	60 mL
-	Plastic bag	1
-	Package insert	1

Storage and Stability (refer to the expiry date on the outer box label)
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. After the first opening the kit should be used within 3 months, the diluted wash buffer can be kept for 4 weeks at 2-8°C.

5.1. Microtiter Strips
12 strips with 8 breakable wells each, coated with mouse monoclonal anti-IgE. Ready-to-use.

5.2. Calibrators (Standards)
1 mL (0 IU/mL), 5x 200 µL (5, 25, 100, 250, 1000 IU/mL), human serum diluted with PBS. Calibrated against the 2nd International Standard 75/502. Addition of 0.1% sodium azide. Ready-to-use.

5.3. Enzyme Conjugate
22 mL, goat anti-human-IgE-HRP, in protein-containing buffer solution. Ready-to-use.

5.4. Substrate
12 mL, TMB (tetramethylbenzidin). Ready-to-use.

5.5. Stop Solution
12 mL, 1 N acidic solution. Ready-to-use.

5.6. Washing Buffer
60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

5.7. Plastic Bag
Resealable, for the dry storage of non-used strips.

5.8. Package insert

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
- Re-usable black lid for covering (Available upon request at Demeditec Diagnostics GmbH)

7. 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.

8. ASSAY PROCEDURE

8.1. Preparation of Reagents

Washing Solution: dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- 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.

8.2. 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 re-usable plate cover 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. Rests 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 re-usable plate cover 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.

9. EVALUATION

9.1. Quantification

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. For a quantitative evaluation the absorptions of the standards are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. Alternatively the use of electronic device is possible. The results can also be calculated with normal programs for automatic data processing, i.e. 4 parameter, spline, logit-log. 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 dilution factor. Do not use the above calibration curve. In the laboratory the standard curve should be established in each assay run.

9.2. Result Interpretation

Normal ranges of Total IgE are dependent on age:

Age	Normal Range [IU/mL]
Newborns	< 1.2
1 – 6 months	< 7.2
7 – 12 months	< 12.7
1 – 5 years	< 60
6 – 9 years	< 155
10 – 15 years	< 199
Adults (Greyzone)	<100 (60-100)

10. ASSAY CHARACTERISTICS

Total Immunoglobulin E 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 %
Measuring Range	5 – 1000 IU/mL

11. 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).

Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
fax 02-92157285
info@listarfish.it
www.listarfish.it