CE IVD 2°C 8°C

Please read instructions for use carefully before starting the assay

Total IgE ALLERG-O-LIQ

Enzyme Immuno Assay for the quantitative determination of Total IgE in human serum and plasma

REF 08101FL Σ 96 Determinations

BACKGROUND

Allergic reactions of the immediate type (Type I allergies) are mediated by allergen specific Immunoglobulin of class E. The normal serum IgE concentration is age dependent with a peak at the age of 6-15 years. Mostly, the occurrence of allergen specific IgE is accompanied by increased titres of total IgE in the blood of the patients. In these cases the titre can increase up to 1000fold. Usually, IgE concentrations are determined in international units per millilitre (IU/mL) wherein 1 IU/mL corresponds to 2.4 ng of IgE. Highest IgE concentrations occur in patients with atopic dermatitis; in which they often reach levels of 50.000 IU/mL. Moreover, increased titres of IgE can be observed in patients with parasitic diseases. Deviations to the normal values have also been described in patients with certain autoimmune disorders.

INTENDED USE

This total IgE Test is intended for the quantitative determination of IgE in human serum or plasma. The results add to the diagnosis of type I allergies. Furthermore, determination of total IgE is recommended in patients with suspected parasitic diseases.

PRINCIPLE

The ALLERG-O-LIQ Total IgE for the quantitative measurement of IgE in human serum or plasma is based on a Sandwich ELISA. During the first incubation step total IgE from the patient sample is captured by anti-human IgE coated to the microwells. By a washing procedure surplus serum components are removed from the well whereas IgE remains bound to the solid phase surface. Detection of total IgE is carried out by a biotin-labelled anti-IgE resulting in anti-IgE/IgE/conjugate complexes. After removing surplus anti-IgE Biotin molecules by washing, streptavidin horseradish-peroxidase (HRP) conjugate is incubated in the wells. The wells are washed again, and the substrate solution 3,3',5,5'-Tetra-Methyl-Benzidine (TMB) is added and incubated, resulting in the development of a blue colour. After stopping the reaction with acid; the colour changes into yellow.

The optical density is proportional to the amount of immobilized immuncomplexes and can be measured spectrophotometrically at 450 nm (reference wave length 620 nm). Calibrators with defined concentrations of IgE (calibrated against WHO 75/502) are assayed simultaneously with the patient samples to generate a calibration curve. Unknown IgE concentrations of the test samples are calculated from this curve.

REAGENTS

Microtiter strips, anti-IgE coated	MICROWELL	12 strips à 8 wells
Anti-IgE Biotin	BIOTIN AB E	1 x 16 mL
Streptavidin HRP conjugate	CONJ HRP STREP	1 x 16 mL
Concentrated Washing Buffer (25x)	WASHBUF B 25x	1 x 50 mL
TMB Substrate	SUB TMB	1 x 16 mL
Stop Solution (0.5M H_2SO_4)	STOP H_2SO_4	1 x 16 mL
Dilution Buffer	DILBUF B	1 x 50 mL
IgE-Calibrator 1 * (0 IU/mL)	CAL 1	1 x 1.5 mL
IgE-Calibrator 2 * (0.7 IU/mL)	CAL 2	1 x 1.5 mL
IgE-Calibrator 3 * (3.5 IU/mL)	CAL 3	1 x 1.5 mL
IgE-Calibrator 4 * (17.5 IU/mL)	CAL 4	1 x 1.5 mL
IgE-Calibrator 5 * (50 IU/mL)	CAL 5	1 x 1.5 mL
IgE-Calibrator 6 * (100 IU/mL)	CAL 6	1 x 1.5 mL
Control low	CONTROL L	1 x 1.5 mL
Control high	CONTROL H	1 x 1.5 mL

*Calibrators are prediluted; concentrations have to be multiplied by 10

MATERIAL NEEDED, BUT NOT PROVIDED WITH THE KIT

10-100 µL and 200-1000 µL Pipettes, Multipette, Pipet tips, vials for diluting the specimen, graduated glass cylinder, microplate-reader, covering foil, microplate-washer (optional), lab watch, distilled water.

SPECIMEN COLLECTION & PREPARATION

Either Serum or Plasma can be used in this test. No additives or preservatives are necessary to maintain the integrity of the specimen.
At 2-8°C specimens are stable for one week. By lengthy storage specimens need to get frozen. Repeated freezing and thawing should be avoided. Frozen specimens should be thawed at room temperature (RT, 20-25°C) and mixed thoroughly by gentle inversion before assaying. Samples should be tested undiluted. The use of haemolysed or lipemic specimens is not recommended.

PREPARATION OF REAGENTS

Allow all reagents to come to RT before use. Microtiterstrips which are not required have to be resealed properly in the provided foil bag containing a desiccant.

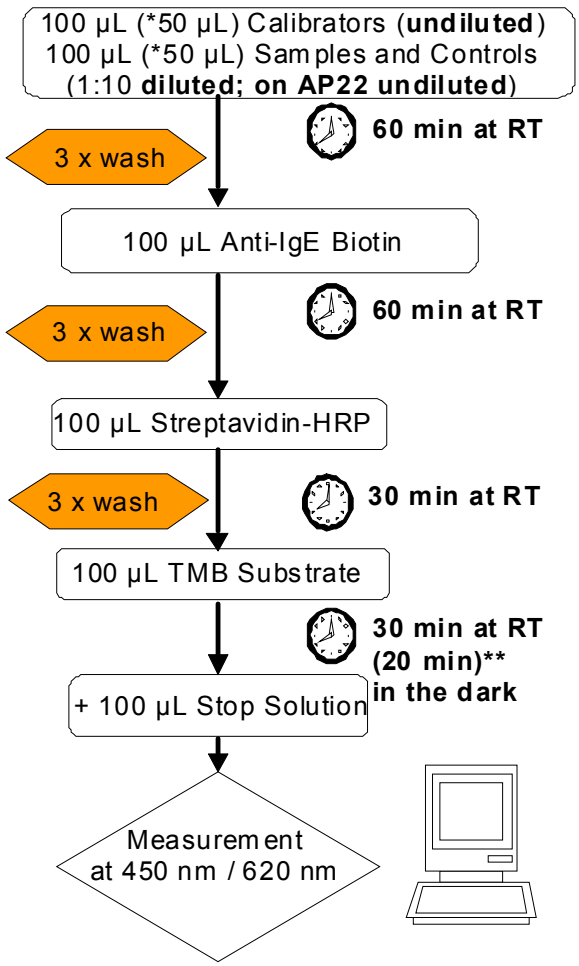
Dilution Buffer: ready to use
Anti-IgE Biotin ready to use
Enzyme conjugate: ready to use
Calibrators: ready to use
Substrate Solution: ready to use
Stop Solution: ready to use

Concentrated Washing Buffer:
The concentrated Washing Buffer has to be diluted 1:25 in distilled water (Example: 2 strips will require 40 mL of Washing Buffer. Therefore 1.6 mL concentrated Washing Buffer has to be diluted to a final volume of 40 mL with distilled water). The resulting Washing Buffer is useable for one week at room temperature.

ASSAY PROCEDURE

1. Prepare a protocol for the assay run. It is recommended to test the Calibrators and Controls in duplicate determination
2. Place the required coated wells into a frame and reseal the aluminium bag with the remaining strips and desiccant properly.
3. Pipette 90 µL of Dilution Buffer in the anti-IgE coated wells (except wells for calibrators and when performed on AP22) and add 10 µL controls and patient samples into their respective wells. Pipette 100 µL of the calibrators to dedicated wells (*50 µL when performed on the AP22).
4. Cover plate and incubate for 60 min at RT (20-25°C).
5. Wash the plate manually or with an appropriate ELISA plate washer 3 times each with 300 µL washing buffer per well. Remove residual liquid by dunking the microplate onto a paper towel.
6. Add 100 µL of Anti-IgE Biotin to each well. Cover and incubate for 60 min at RT.
7. Repeat washing procedure as described in step 5.
8. Pipette 100 µL of Streptavidin HRP conjugate into each well, cover the microplate and incubate for 30 min at RT.
9. Repeat washing procedure as described in step 5.
10. Add 100 µL of TMB Substrate to each well, cover the plate and incubate for 30 min (** 20 min on the AP22) at RT in the dark.
11. Pipette 100 µL of Stop Solution in the same order as the substrate to each well. It is recommended to mix the solution in the wells by carefully knocking on the frame. Read OD at 450 nm (reference wave length 620 nm) using an appropriate microplate reader and calculate the results as described on page 3.

TEST SCHEME Total IgE ALLERG-O-LIQ



* 50 µL when performed on the AP22
** Incubation time on AP22 ELISA Processor

DR. FOOKE
Laboratorien GmbH Tel.: 0049-2131-2984-0
Habichtweg 16 Fax: 0049-2131-2984-184
4 1 4 6 8 Neuss
E-mail:information@fooke-labs.de
Internet: www.fooke-labs.de

CALCULATION OF RESULTS

It is recommended to use validated software for the calculation of the results. For manual calculation, the mean OD [Δ 450 nm – 620 nm] values are calculated from the Calibrators and Controls. Generate a graph from the mean OD values of the four Calibrators on half logarithmic paper (Abscissa: log IU IgE/mL; Ordinate: linear OD Δ 450 nm – 620 nm) to create a standard curve. The IgE concentration of the patient sample is determined on the basis of this standard curve. The OD is mapped on the Ordinate and the result in IU/mL can be read out on the Abscissa. The standard curve and the controls should be in the acceptance range given in the Quality-Control-Certificates delivered with the kit. Otherwise, the test conditions should be verified and the test should probably be repeated.

EXAMPLE CALIBRATOR CURVE

Calibrator Concentration (IU/ml)	OD 450 nm Mean (n=22)	Acceptance range OD 450 nm
0	0.039	0.027 - 0.051
7	0.155	0.108 - 0.201
35	0.564	0.395 - 0.733
175	1.823	1.276 - 2.370
500	2.648	1.854 - 3.443
1000	3.189	2.233 - 4.146

EXPECTED VALUES

It is recommended, that expected values for given populations should be determined by each laboratory over a period of time and in a statistically significant number of assays before clinical significance is attached to the results of the assay. The values given below (Wittig *et al.)* can be used as a guideline for the own results.

Age (years)	n	Mean (IU/mL)	Mean + 1 SD (IU/mL)
1-2	29	20	64
3-5	31	35	119
6-15	45	51	150
16-20	59	38	123
21-30	114	27	100
31-40	38	34	113
>40	109	34	114
Total	425	32	108

MEASURING RANGE

IgE concentrations between 5 IU/mL and 1000 IU/mL can be determined by this ELISA. Specimens with concentrations higher than 1000 IU/mL should be retested in an appropriate dilution to determine the accurate IgE concentration.

Precision

Variability and Reproducibility

1. ***Intra***-Assay Variability
   (1 test in 4fold determination)

Specimen	Mean IU/mL	CV (%)
1 (n=4)	339.7	3.9
2 (n=4)	41.5	1.5
3 (n=4)	9.4	8.8

2. ***Inter***-Assay Variability
   (2 tests in duplicate)

Specimen	Mean IU/mL	CV (%)
1 (n=4)	331.0	3.0
2 (n=4)	105.3	0.5
3 (n=4)	34.1	1.7

LINEARITY

Linearity of this quantitative immunoassay has been ensured using 5 randomly selected patient sera by serial dilutions showing a linear behaviour in five consecutive dilution steps (≤ ± 20%). Due to the heterogeneity of human material different results may be possible.

SPECIFICITY

In physiological concentrations no cross-reactivity to other Ig-classes could be observed using this IgE test.

AGREEMENT TO REFERENCE METHOD

The correlation coefficient was found at $R^2 = 0.96$ (to Total IgE HRP EIA, REF: 08102CP) and $R^2 = 0.88$ (ImmunoCAP®).

LITERATURE

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 Immunol* 1966, **97**:75-85.

2. Hamilton R: **Radioimmunoassay in the Assessment of Allergic Disease**, *Ligand Quarterly* 1979, **2**:13-19.

3. Johansson S, Bennich H, Berg T: "The Clinical Significance of IgE", *Progress in Clin Immunol* 1972, 1.

4. Kjellman, M.: **Immunoglobulin IgE and Atopic Allergy in Childhood.** *Linkpoing University Medical Dissertations* No 36 (1976).

5. Wittig, H., Bellot, J., Fillippi, I. and Royal, G.: **Age-related Serum IgE Levels in Healthy Subjects and in Patients with Allergic Disease.** *J Allergy Clin Immunol* 1980, **66**:305-313.

6. Gleich G, Averbeck A, Swedlund H: **Measurement of IgE in Normal and Allergic Serum by Radioimmuno-assay**. *J Lab and Clin Med* 1971, **77**:690-698.

PRECAUTIONS FOR USERS

1. In compliance with article 1 paragraph 2b European directive 98/79/EC the use of *in-vitro* diagnostic medical devices is intended to secure suitability, performance and safety of the product by the manufacturer. Therefore the test procedure, information, precautions and warnings stated in the instructions for use have to be followed strictly. The kit has only to be used as described on page 1 (intended use).
2. The test must be performed according to this instruction, which contains all necessary information, precautions and warnings. The use of the test kit with analyzers and similar equipment has to be validated. Any change in design, composition of the test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes resulting in false results and other incidents. The manufacturer is not liable for any results obtained by visual analysis of patient samples.
3. The kit is intended for use by trained and qualified professionals carrying out research or diagnostic activities only. Pregnant women should not perform the test.
4. Laboratory equipment has to be maintained according to the manufacturer's instructions and must be tested for its correct function before use.
5. For *in-vitro* diagnostic use only. Use only once. Do not use components exceeding the expiry date. Do not combine reagents of other suppliers or kit components of different lots (unless specified on page 1) with this kit.
6. Do not use kit components when the package of the component is damaged. Please check all solutions prior to use for microbiological contamination. Cap vials tightly immediately after use to avoid evaporation and microbiological contamination. Do not interchange screw caps of the reagent vials.
7. The kit was evaluated for use at the temperatures specified in the Testing scheme (see page 2). Higher or lower temperatures may result in values not meeting the quality control ranges.
8. The washing procedure is absolutely important. Improper washing will cause erroneous results. It is recommended to use a multichannel pipette and an automated washer.
9. To avoid cross-contamination and false-positive results it is recommended to perform all pipetting steps properly. Use only clean pipette tips, dispensers and lab ware.
10. Test components based on human serum were tested using a CE marked method for the presence of antibodies against HIV 1 / HIV 2, Anti-HBc, and Anti-HCV as well as for hepatitis antigen HBsAg and were found to be negative. Nevertheless, material based on human serum should be handled as potentially infectious (BIOHAZARD).
11. Some kit components may contain bovine serum albumin, of which according to the manufacturer no infectious potential is known. Due to the eventual occurrence of undetectable infectious agents we recommend to handle any product of animal origin as potentially infectious.
12. The following safety rules should be followed with all reagents:
    - Do not get in eyes, on skin, or on clothing (P262). Do not breathe spray (P260). Pipetting should never be done by mouth, but with suitable pipetting devices.
    - IF SWALLOWED: rinse mouth. Do NOT induce vomiting (P301/330/331)
    - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower (P303/361/353).
    - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing (P303/340).
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305/351/338)
    - Don't eat, drink or smoke while performing the test. Keep away from food, feed and beverage.
    - Wear protective gloves/protective clothing/eye protection (P280). Wash hands thoroughly after handling (P264) and care for your skin.
    - Material safety data sheet is available on request.
13. Stop Solution causes severe skin burns and eye damage (H314).
14. TMB in high concentrations may be potentially mutagenic. Due to the low concentration of TMB in this substrate solution a mutagenic effect can be ruled out, if it is properly used.
15. The preservatives (Bronidix, Thimerosal, Azid) are toxic to aquatic life, but their concentration is not hazardous to environment anymore. On disposal, flush large volumes of reagents with plenty of water. Thimerosal (WashBuf B) may cause damage to organs through prolonged or repeated exposure (H373):
16. Waste containing serum must be collected in separate containers containing an appropriate disinfectant in sufficient concentration. This material has to be treated according to national biohazard and safety guidelines or regulations.
17. We refer to the national regulations of medical devices regarding *in-vitro* diagnostic test kits.

D R . F O O K E
Laboratorien GmbH Tel.: 0049-2131-2984-0
Habichtweg 16 Fax: 0049-2131-2984-184
4 1 4 6 8 Neuss
E-mail:information@fooke-labs.de
Internet: www.fooke-labs.de

LOT	CE	IVD	2°C 8°C		REF					Σ		
Lot-Number	European conformity	For *in-vitro* diagnostic use	Temperature Limit	Use before	Catalogue Number	Consult instructions for use	Refer to accompanying documents	Do not use when package is damaged	Do not Re-use	Sufficient for <n> tests	Manufactured by	Biohazard