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DR. FOOKE LABORATORIEN GmbH

Please read instructions for use carefully before starting the assay

Total IgE-HRP EIA

Enzyme Immuno Assay for the quantitative determination of Total IgE in human Serum or Plasma

REF 08102CP Σ 96 Determinations

BACKGROUND

Allergic reactions of the immediate type (Type I allergies) are mediated by allergen specific Immunoglobulin of class E (IgE). 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 titers of total IgE in the blood of the patients. In these cases the titer can increase up to 1000fold. Usually, IgE concentrations are determined in international units per millilitre (IU/mL) wherein 1 IU 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 titers of IgE can be observed in patients with parasitic diseases. Abnormal titers have also been described in certain autoimmune disorders.

INTENDED USE

The Total IgE-HRP EIA 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 Total IgE-HRP EIA for the quantitative measurement of IgE is based on a Sandwich ELISA performed in microplates. During the first incubation step total IgE from the patient sample is capture by anti-human IgE coated to the microwells. By a washing procedure surplus serum/plasma components are removed from the wells whereas IgE remains bound to the solid phase surface.

Detection of bound IgE is carried out with an anti-human IgE horseradish-peroxidase (HRP)-labelled antibody forming anti-IgE/IgE/ conjugate complexes. 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 enzymatic reaction with sulphuric acid (H_2SO_4) the colour changes into yellow. The optical density (OD) of the coloured product is measured spectrophotometrically at 450 nm (reference wave length 620nm). The IgE concentration of the patient sample is proportional to the OD.

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.

KIT COMPONENTS

Components	**REF**	**08102CP**
Microtiter strips, anti-IgE coated	MICROWELL	12 strips à 8 wells
Anti-IgE HRP-Conjugate	CONJ HRP E	1 x 12 mL
Concentrated Washing Buffer (50x)	WASHBUF C 50x	1 x 30 m
TMB Substrate	SUB TMB	1 x 12 mL
Stop Solution (0.5 M H_2SO_4)	STOP H_2SO_4	1 x 12 mL
Dilution Buffer	DILBUF A	1 x 12 mL
IgE-Calibrator 1 (5 IU/mL)	CAL 1	1 x 0.5 mL
IgE-Calibrator 2 (20 IU/mL)	CAL 2	1 x 0.5 mL
IgE-Calibrator 3 (50 IU/mL)	CAL 3	1 x 0.5 mL
IgE-Calibrator 4 (100 IU/mL)	CAL 4	1 x 0.5 mL
IgE-Calibrator 5 (200 IU/mL)	CAL 5	1 x 0.5 mL
IgE-Calibrator 6 (1000 IU/mL)	CAL 6	1 x 0.5 mL
IgE-Control low	CONTROL L	1 x 0.5 mL
IgE-Control high	CONTROL H	1 x 0.5 mL

MATERIAL NEEDED, BUT NOT PROVIDED WITH THE KIT

10-100µL and 200-1000µL pipettes, Multipette, pipette 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.
Specimens should be stored at 2-8°C and assayed within 48 hours after collection. If the assay cannot be performed within 48 hours or if the specimen has to be shipped, cap the specimen and keep it 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 have to be diluted 1:6. We recommend to dilute the samples directly in the anti-IgE coated microwells (pipette first 100 µL Dilution buffer, then 20 µL Sample). The use of hemolytic 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.

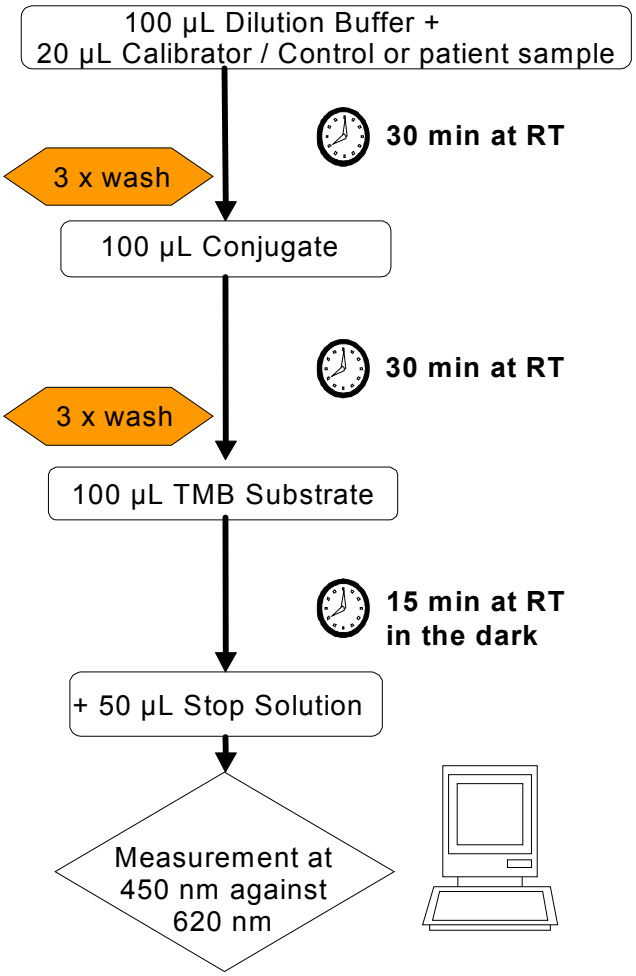
Dilution buffer: ready to use
Enzyme conjugate: ready to use
Substrate Solution: ready to use
Stop Solution: ready to use
Calibrators and Controls: ready to use

Concentrated Washing Buffer:
The concentrated Washing Buffer has to be diluted 1:50 in aqua bidest. (Example: 2 strips will require 40 mL of Washing Buffer. Therefore 800 µL concentrated Washing Buffer have to be diluted to a final volume of 40 mL with aqua bidest.). The resulting Washing Buffer is stable for one week at RT.

ASSAY PROCEDURE

1. Create a pipetting schedule. It is highly recommended to test the Calibrators and Controls as well as all patient samples in duplicate. To minimize time delays when pipetting, all needed samples should be provided before starting the test. It is recommended to complete pipetting for one plate within 10 min.
2. Place the required coated wells into a frame and reseal the aluminium bag with the remaining strips and desiccant properly.
3. Pipette 100 µL of Dilution Buffer to each well and add 20 µL of appropriate calibrators, controls and patient samples into their respective wells.
4. Cover plate and incubate for 30 min at RT.
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 100µL of anti-IgE HRP conjugate to all wells. Cover plate and incubate for 30 min at RT.
7. Repeat washing procedure as described in step 5.
8. Add 100 µL of TMB Substrate to each well, cover the plate and incubate for 15 min at RT in the dark.
9. Pipette 50 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. After 5 min 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-HRP EIA



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

The mean OD [Δ 450 nm – 620 nm] values are calculated from the Calibrators, Controls and patient samples. 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 mean 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
5	0.074	0.056 - 0.130
20	0.217	0.163 - 0.271
50	0.438	0.329 - 0.548
100	0.753	0.565 - 0.941
200	1.076	0.907 - 1.345
1000	2.076	1.557 - 2.595

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 dedicated to the results of the assay. The values given below 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

SD = Standard deviation

MEASURING RANGE

IgE concentrations between 5 IU/mL and 1000 IU/mL can be determined by this ELISA. Samples 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)	25.8	4.5
2 (n=4)	141.1	4.4
3 (n=4)	397.6	5.3

2. ***Inter***-Assay Variability
   (3 tests/4 determinations each)

Specimen	Mean IU/mL	CV (%)
1 (n=12)	27.7	6.4
2 (n=12)	148.5	6.1
3 (n=12)	415.2	9.9

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

The Total IgE-HRP EIA specifically detects human IgE molecules. In physiological concentrations no cross-reactivity to other Immunoglobulins such as IgA, IgD, IgM and IgG has been observed.

LITERATURE

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5. Wittig H, Bellot J, Fillippi I, 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., and Swedlund, H.: **Measurement of IgE in Normal and Allergic Serum by Radioimmuno-assay**. *J Lab and Clin Med* 1971, **77**:690-698.

7. Arbeitsgruppe der Deutschen Diagnostika Gruppe e.V. (DDG): **Gute Labordiagnostische Praxis GLDP, Konzept einer „Guten Labordiganostischen Praxis".** *Clin Lab* 1999; 45: 569-580.

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, 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. (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- Number	European conformity	For *in-vitro* diagnostic use	Temperature Limit	Use before	Catalogue Number	Consult instructions for use	Refer accompanying documents	Do not use when package is damaged	Do not Re-use	Sufficient for <n> tests	Manu-factured by	Biohazard