

Please read instructions for use carefully before starting the assay

Specific IgE REAST

Reversed Enzyme Immuno Assay for the quantitative determination of specific IgE in human Serum or Plasma with biotinylated Allergens

REF	0520960FL	Σ	96 Determinations
REF	0524800FL	Σ	480 Determinations

BACKGROUND

The worldwide frequency of allergies has increased significantly over the past decades. The term allergy is often used for type I hypersensitivity reactions (immediate type reactions), whose symptoms generally occur within 30-60 min after contact with the allergen. The most frequent symptoms are: hay fever (rhinitis), conjunctivitis, hives (urticaria), allergic asthma and as the most dangerous manifestation anaphylaxis (the anaphylactic shock).

The allergens causing type I hypersensitivity reactions are mostly proteins derived from the natural environment e.g. plant pollen, animal hair, food, mites and insect venoms.

The characteristics of type I allergies is the involvement of allergen specific immunoglobulins (antibodies) of class E (sIgE). Hence, the detection of sIgE is an important tool of modern allergy diagnostics.

INTENDED USE

The Specific IgE REAST is intended for the quantitative determination of sIgE in human serum or plasma. The results add to the diagnosis of type I allergies.

PRINCIPLE

The Specific IgE REAST for the quantitative measurement of IgE 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. During the next incubation step biotinylated allergen is added and incubated in the microwells. After a further washing step detection of bound allergen is carried out with a streptavidin/peroxidase (HRP)-conjugate forming complexes consisting of specific IgE/biotinylated allergen/HRP-conjugate.

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 acid the colour changes into yellow. The optical density (OD) of the coloured product is measured spectrophotometrically at 450 nm (reference wave length 620 nm). The sIgE concentration of the patient sample is proportional to the optical density.

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

Enzyme kit	REF	0520960FL 0524800FL
Microtiterstrips, anti-IgE coated	MICROWELL	1 Plate 5 Plates
Streptavidin HRP-Conjugate	CONJ HRP STREP	1 x 16 mL 1 x 55 mL
Washing Buffer Concentrate (25 x)	WASHBUF B 25x	1 x 50 mL 1 x 110 mL
TMB Substrate	SUB TMB	1 x 16 mL 1 x 55 mL
Stop Solution (0.5 M H ₂ SO ₄)	STOP H₂SO₄	1 x 16 mL 1 x 55 mL

MATERIAL NEEDED BUT NOT INCLUDED IN THIS KIT

1. Reference unit	REF	07050FL
Anti-IgE Biotin conjugate	BIOTIN AB E	1 x 10 mL
Calibrators (0.35, 0.7, 3.5, 17.5, 50, 100 IU/mL)	CAL (1-6)	6 x 1.5 mL

2. Biotinylated allergens	REF	Allergen code + FL
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3. Controls	REF	07005/07006
Positive Control	CONTROL +	1 x 1 mL
Negative Control	CONTROL -	1 x 1 mL

OTHERS

10-100 µL and 200-1000 µL pipettes, Multipipette, 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.

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. The use of haemolysed or lipemic specimens is not recommended.

PREPARATION OF REAGENTS

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

Substrate Solution: ready to use

Stop Solution: ready to use

Calibrators and Controls: ready to use

Enzyme conjugate: ready to use

Anti IgE Biotin ready to use

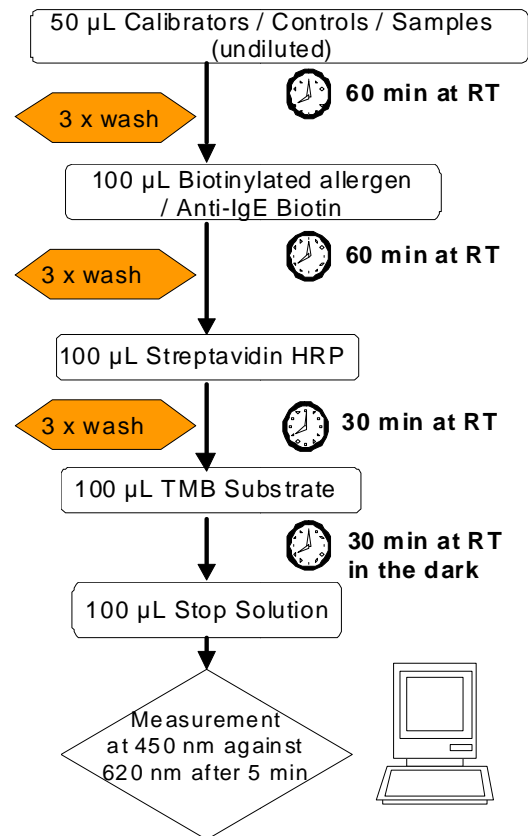
Concentrated Washing Buffer: The concentrated Washing Buffer has to be diluted 1:25 in distilled water (Example: 2 strips will require 50 mL of Washing Buffer. Therefore 2 mL concentrated Washing Buffer have to be diluted to a final volume of 50 mL with distilled water). The resulting Washing Buffer is useable 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.
2. Place the required coated wells into a frame and reseat the aluminium bag with the remaining strips and desiccant properly.
3. Pipette 50 µL of undiluted calibrators, controls and patient samples into their respective wells.
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 (overflow) 3 times each with 500 µL washing buffer per well. Remove residual liquid by dunking the microplate onto a paper towel.
6. Add 100 µL of the respective allergen solution (for patients and controls) and of the anti-IgE Biotin conjugate (for standards) according to the pipetting scheme, cover plate 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 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 values after 5 min at 450 nm (reference wave length 620 nm) using an appropriate microplate reader and calculate the concentrations of controls and samples as described on page 3.

TEST SCHEME SPECIFIC IgE REAST Manual Processing



Device-specific settings apply for fully automated processing.

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CALCULATION OF RESULTS

The average optical density (OD) [Δ 450 nm - 620 nm] values are calculated from the calibrators, controls and patient samples. Generate a graph from the average OD values of the six calibrators on half logarithmic paper (Abscissa: log IU IgE/mL; Ordinate: linear optical density Δ 450 nm - 620 nm). The sIgE concentration of the patient sample is determined on the basis of the standard curve. The average optical density is mapped on the Ordinate and the result in IU/mL can be read out on the Abscissa. The calibrator 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
0.35	0.106	0.074 - 0.138
0.7	0.161	0.113 - 0.209
3.5	0.568	0.398 - 0.738
17.5	1.619	1.133 - 2.105
50.0	2.558	1.791 - 3.325
100.0	3.015	2.111 - 3.920

EVALUATION OF THE RESULTS

Using the calibration curve the sIgE concentrations of samples are calculated either in REAST-Classes and/or in international units per millilitre (IU/mL) and evaluated as follows:

Class	IU/mL	spec. IgE concentr.
6	> 100	extremely positive
5	50 - 100	very strong positive
4	17.5 - 50	strong positive
3	3.5 - 17.5	clearly positive
2	0.7 - 3.5	moderate
1	0.35 - 0.7	low
0	< 0.35	not detectable

RESULTS TO BE EXPECTED

The clinical relevance of a positive test report varies significantly between individual allergens. Therefore, 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 can be used as a guideline for the own results.

MEASURING RANGE

This ELISA detects sIgE concentrations between 0.35 IU/mL and 100 IU/mL. Specimens with higher sIgE concentrations should be diluted and retested to determine the exact sIgE content.

PRECISION

Variability and Reproducibility

1. Intra-Assay Variability

Specimen	Mean IU/mL	CV (%)
1 (n=10)	8.6	2.0
2 (n=7)	94.6	6.6

2. Inter-Assay Variability

Specimen	Mean IU/mL	CV (%)
1 (n=20)	8.5	3.8
2 (n=16)	95.8	6.7

LINEARITY

5 randomly selected patient sera displayed a linear behaviour in three consecutive dilution steps ($< \pm 20\%$). Due to the heterogeneity of human serum or plasma samples different results may be possible.

SPECIFICITY

The Specific IgE REAST specifically detects human sIgE molecules. In physiological concentrations cross-reactivity to other Immunoglobulins such as IgA, IgD, IgM and IgG has not been observed.

LIMITATION OF THE METHOD

The Specific IgE REAST has the following limitations:

- A negative test result does not exclude a Type I allergy
- The test result has to be considered in the context of the patient's history
- For test samples with high amount of total IgE (> 1000 IU/mL) the measured concentrations of sIgE may be lower than the effective values
- By using allergen mixtures only a semi-quantitative determination is possible

LITERATURE

1. Johansson S, Bennich H and Berg T: **The Clinical Significance of IgE.** *Progress in Clin Immunol*, 1972, 1.
2. Kjellman M: **Immunoglobulin IgE and Atopic Allergy in Childhood.** *Linköping University Medical Dissertations* No 36 (1976).
3. Kleine-Tebbe J, Breuer K, Lepp U, Vieths S, Worm M, Kunkel G, Wahn U und Lau S: **Spezifische IgE-Werte gegen Aero- und Nahrungsmittelallergene im Vergleich: Dr. Fooke ALLERG-O-LIQ und Pharmacia CAP-System.** *Allergologie* 2004, 4:129-134.
4. Arbeitsgruppe der Deutschen Diagnostika Gruppe e.V. (DDG): **Gute Labordiagnostische Praxis GLDP, Konzept einer „Guten Labordiagnostischen Praxis“.** *Clin Lab* 1999, 45:569-580.
5. Sun B, Mahler M, Mei J, Jing L, Zhong N: **Allergen-specific IgE to inhalant and food allergens and total IgE values in China – Comparison between two commercial immunoassays: Dr. Fooke ALLERG-O-LIQ versus Phadia ImmunoCAP® System.** *J Invest All Clin Immunol* 2008, 18:319-321.

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.



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Lot- Number	European conformity	For <i>in-vitro</i> 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	Bio-hazard