For in-vitro use only! Instructions for use Omega Diagnostics GmbH

Allergozyme® Total IgE

CE REF 36040000

Enzyme immunoassay for the quantitative determination of total IgE in human serum or plasma.

Micro-titration plate version for 96 tests

1. Intended use

Enzyme immunoassay for the quantitative determination of total IgE in human serum or plasma. Determination of total IgE with this test kit is validated in association with the Omega Diagnostics test system and the determined performance data have been established for the Omega Diagnostics test systems. For the use with other test systems the validation has to be performed by the user. Use is restricted to qualified specialists, who have been specially instructed and trained in processes which are carried out with the use of IVDs.

2. Introduction

Immunoglobulin E is a serum protein and the main carrier of reactive activity of type I allergic reactions (immediate type).
IgE circulates in blood. The surface of mastocytes and IgE which is bound to basophil granulocytes are responsible for the clinical symptoms of the type I reaction. Binding occurs on the Fc component of the IgE molecule. If an allergen comes into contact with the corresponding (specific) cell-bound IgE, pro-inflammatory mediators and enzymes (e.g. histamine) are released. The total IgE test measures all of the specific IgE variants in the serum and is therefore a collective test which provides interpretative assistance for the diagnosis but e.g. does not give evidence of sensitivity to a specific allergen. Therefore, the results of the determination of the total IgE in the serum should only form part of a diagnostic concept, which also includes a detailed history and skin and provocation tests.

3. Test Principle

The quantitative determination of the circulating total IgE in human serum is carried out by means of a non-competitive enzyme immunoassay. The solid phase consists of a micro-titration well, on the inner surface of which an anti-IgE is adsorptively bound. In the first step, the patient serum or plasma and the diluents solution are pipetted into the well. A binding of the total IgE to the anti-IgE bound to the solid phase takes place. Unbound serum is removed in a washing stage. In the second step an enzyme-labeled anti-human IgE is pipetted into the well. A binding of the labeled anti-human IgE to the total IgE then takes place. Unbound labelled anti-human IgE is removed in a washing stage. The quantity of bound and labeled anti-human-IgE is proportional to the quantity of the total IgE in serum or plasma. In the next step a substrate solution (p-nitrophenyl phosphate) is added. Due to the activity of alkaline phosphatase, a coloured solution is obtained. At the end of the incubation period the enzyme reaction is terminated with a stop solution. The extinctions of the coloured solutions are measured with a photometer. Evaluation is performed by means of a standard curve consisting of the extinction values of the measured standard wells.

4. Contents of the Total IgE test kit

1. CONJ Conjugate: 1 bottle with 12 ml monoclonal anti-human-IgE (mouse), conjugated with alkaline phosphatase in a buffered protein solution; preservation agent: 0.02% sodium azide. Differences in colour do not affect the efficacy of the conjugate.
2. WASH 20x Washing solution (concentrate): 1 bottle with 50 ml concentrated sodium chloride solution with Tween 20; preservation agent: 0.05% sodium azide (for production of the washing solution see 10.2).
3. SUBS Substrate solution: 1 bottle with 12 ml p-nitrophenyl phosphate (PNPP).
4. STOP Stop solution: 1 bottle with 6 ml 1 M sodium hydroxide solution.
5. DIL AS Diluent solution: 1 bottle with 12 ml diluent solution; Preservation agent: 0.05 % sodium azide.
6. MTP Micro-titration plate (snap-off): 1 micro-titration plate, with 12 strips of 8 wells, coated with anti-human IgE (mouse).
7. CAL SERUM Calibration system: 5 bottles, each with 0.3 ml human IgE calibrated against WHO IRP 11/234. Preservation agent: 0.02% sodium azide.
   The standards are filled in increasing concentrations:
   - CAL SERUM 1 Calibrator 1 = 5 IU/ml;
   - CAL SERUM 2 Calibrator 2 = 50 IU/ml;
   - CAL SERUM 3 Calibrator 3 = 100 IU/ml;
   - CAL SERUM 4 Calibrator 4 = 200 IU/ml;
   - CAL SERUM 5 Calibrator 5 = 1000 IU/ml.

5. Additional materials and devices

Materials and equipment:

- Micro-pipette with disposable tips 10 µl
- Manual hand dispenser e.g. Eppendorf Multipette with Combitips 2.5 and 5 ml
- Measuring cylinder 2000 ml
- Micro-titration plate cover
- Disposable gloves
- Distilled water
- Stop watch
- Printer
- Micro-titration plate photometer 405 nm (e.g. TECAN Spectra or TECAN Sunrise)
- Incubator (37 °C)
- Washer for micro-titration plates (e.g. TECAN-Columbus Washer or TECAN Hydroflex)

6. Limitations of the procedure

- Reliable and reproducible results can only be obtained if the test is performed correctly (see test procedure, Section 10).
- If several micro-titration plates are used in a test, the incubation times of the individual plates must be observed.
- The clinical diagnosis should not simply be based on the sole evidence of increased or decreased total IgE, but rather on other clinical data and test results. The in-vitro determination of total IgE should never be used as the sole diagnostic decision criteria for starting a hypersensitisation treatment. In addition, skin tests, other in-vitro tests (e.g. specific IgE) and – if possible - challenge tests should be performed to provide evidence of clinical relevance (see reference 1).
- Negative or low total IgE results may occur if, among other things:
  - the symptoms are not caused by IgE;
  - the sample was taken before the body was able to produce antibodies against the antigen;
  - If the IgE level has returned to a low level a long time after sensitisation.
- Identical results with different patients do not cause the same reaction, as this varies according to the individual.
- Positive results for total IgE in-vitro test need not automatically cause the same clinical symptoms.

Increased total IgE values can also be an indication of an atopic disposition. In special cases, the total IgE can be used as a supplementary diagnostic method for atopically associated disorders (e.g. farmer's lung, vasculitides etc.) as was as for the diagnosis of hereditary and acquired immune deficiencies (hyper-IgE syndrome or T-cell defects) (see Reference 1).
In combination with the determination of specific IgE, total IgE is used as an additional parameter for the assessment of the specific IgE titre. It can neither confirm nor exclude a specific sensitisation. Total IgE is only suitable to a limited extent for 'atopic screening'

7. Specific performance data

- Analytical specificity	Cross-reactivity with IgG Not to be expected up to 25 mg IgG/ml
- Reproducibility (Inter-Assay [basic IU/ml])	< 12 %
- Repeatability (Intra-Assay [basic IU/ml])	< 7 %
-Recovery accuracy (With 3 serums, each with 3 measuring points in the range 55 – 440 IU/ml; CV < 20%)	109,4 %
- Lowest detection level	< 5 IU/ml
- Measurement range	5 – 1000 IU/ml
- Traceability of the total IgE calibrators:	WHO IRP 11/234

Further performance criteria can be provided on request. Please contact Omega Diagnostics In-vitro Marketing.

8. Relevant interferences

Icterus	0 – 0.1 mg/ml bilirubin – no impairment
Haemolysis	0 – 8 mg/ml haemoglobin – no impairment
Lipidaemia	0 – 5 mg/ml triglyceride – no impairment

9. Preparation and storage of specimen

Serum and plasma which has been stored for up to 5 days at 2 to 8 °C can be used. If the test is not performed within this time, it is recommended that the sample is frozen at – 20 °C (storage time at –20 °C at least 6 months). Avoid repeated thawing and freezing!

10. Test procedure

1. Before starting the test, all components must be heated to room temperature (RT, 20 to 25 °C).
2. Preparation of the washing solution: 50 ml of the washing solution concentrate to 1000 ml with distilled water and mix thoroughly. After dilution the solution can be stored for 24 hours at room temperature if thoroughly cleaned vessels are used.
3. Production and distribution scheme for calibrators and examination samples.
   Please note: A double determination of the calibrators is necessary.
4. Pipette 10 µl of standard serums 1 – 5 and then 10 µl of the serum or plasma samples into the wells provided (on the bottom of the wells). Well A1 (blank value) remains empty.
5. Pipette 100 µl of diluent solution into all of the wells (incl. blank value).
6. Cover the micro-titration plate and incubate for 1 hour at 37 °C
7. Wash the wells of the micro-titration plates either with the automatic washer (4x) or a manual washer (5x, please observe the operating instructions!) Only washing procedures approved by Omega Diagnostics must be used.
8. Pipette 100 µl of conjugate into all of the wells (incl. blank value).
9. Cover the micro-titration plate and incubate for 1 hour at 37 °C
10. Wash the wells of the micro-titration plates either with the automatic washer (4x) or with a manual washer (5x, please observe the operating instructions!) Only washing procedures approved by Omega Diagnostics must be used.
11. Pipette 100 µl substrate solution into all of the wells (including blank value).
12. Cover the micro-titration plate again and incubate without exposure to light for 1 hour at 37°C.
13. In the same manner and sequence as for pipetting of the substrate solution, now add 50 µl of stop solution to all of the wells (incl. blank value).
14. After stopping the reaction with the stop solution, the colour complex must be measured within 30 minutes. Place the micro-titration plates with the stopped coloured solution in the photometer. The measurement is performed with 405 nm.
15. For each test run, values for quality control (positive control) should also be measured.

Warning! If significant changes are made to the test procedure (e.g. time, sequence, temperature etc.) or if significant impairment of the analysis performance is seen, even with correct use (e.g. control serum values out of specifications, serious differences in double values etc.) the values which are obtained must not be used. A check of the system or the procedure is essential before continuing work. In case of doubt please contact the specialists at Omega Diagnostics.

11. Calculation

With Omega Diagnostics devices calculation of the reference curve and the evaluation of the measurement results are carried out automatically.

Total IgE standard	Concentration
1	5 IU/ml
2	50 IU/ml
3	100 IU/ml
4	200 IU/ml
5	1000 IU/ml

The standard curve can be calculated manually by entering the extinctions determined for the calibrators against the standard unit values on semi-logarithmic graph paper and connecting the individual points with a ruler. This standard curve is used to determine the values of the serum or plasma samples.

12. Normal values

In adults, values in excess of 100 IU/ml may indicate a possible atopic allergy. See also Section 6 "Limitations of the procedure" and Reference 1. For normal values for children, see Reference 3.

13. Warnings and precautions

The following rules must be observed:

1. The relevant safety regulations must be observed when handling the test components.
2. References and examination samples are potentially infectious substances. Suitable agents or methods must be used to disinfect contaminated areas. The references do not show any reactivity to HB_SAg (Hepatitis B Surface Antigen), HCV and HIV- 1/2.
3. The stop solution contains sodium hydroxide. Wear protective gloves / protective clothing / eye protection / face protection. In case of contact with the skin (or hair): take off all contaminated clothing immediately. Wash or shower the skin with water. In case of contact with the eyes: carefully rinse with water for several minutes. If possible, remove any contact lenses. Continue rinsing. Inform the poison centre or doctor immediately. Wash contaminated clothing before wearing it again.
4. Smoking, eating and drinking are prohibited in the laboratory. Do not ingest!
5. Do not suck the pipette with your mouth!
6. Close all reagents after use. The closures must not be mixed up.
7. Do not use damaged or contaminated kit components.
8. Avoid cross-contamination when pipetting!
9. Test components from different batches must not be mixed.
10. Reagents must not be used after the expiry date.
11. Reference samples and kit controls must be included with every assay array performed to ensure correct results.
12. The functionality and accuracy of the equipment used (pipettes, photometer etc.) must be checked at regular intervals. Observe the manufacturer's instructions!
13. Reagents and chemicals must be handled and disposed of according to the applicable regulations.

List of supplied substances which may require special treatment for disposal:

- Conjugate (sodium azide <0.1% w/w CAS 26628-22-8;
  bovine serum albumin CAS 90604-29-8)
- Washing solution (sodium azide <0.1 % w/w
  CAS 26628-22-8)
- Substrate (p-Nitrophenyl phosphate
  CAS 4264-83-9)
- Stop solution (sodium hydroxide 1 M CAS 1310-73-2)
- Standards (sodium azide <0.1% w/w
  CAS 26628-22-8;
  human serum)

14. Quality control

- *Internal quality control*
  It is recommended that for each test set at two positive controls (if available) and patient samples the test. Omega Diagnostics provides such control samples. The product information of the control samples contains the normal ranges for this test. If the positive control is within the normal ranges, it can be assumed that the test method is functioning adequately.
  It is recommended that quality control records are kept.
- *External quality control*
  Participation in external quality controls (ring tests) is recommended. Here, samples with unknown analytical concentrations are not known to the laboratory participating in the external quality control are sent by a ring test provider. After collection of the results, the ring test provider evaluates and assesses the results from all senders. Details must be obtained from the ring test provider. Please contact Omega Diagnostics or your in-vitro sales representative.

15. Storage of the test kit

2 to 8 °C

16. Expiry date

The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Expiry date is the last day of the month on the bottle and the kit label. Do not use reagents after the expiry date.

17. References

1. Ring J., 1992, Angewandte Allergologie [Applied Allergology], MMW Verlag, München
2. R. Wahl, R. Krause: Methoden der In-vitro-Allergiediagnostik und deren Stellenwert unter Berücksichtigung ihrer technischen Aspekte. [Methods of in-vitro allergy diagnostics and their importance, in consideration of their technical aspects] Allergologie 33/3, 2010 , 121-133.
3. Renz H., et al.:In-vitro-Allergiediagnostik, Leitlinie der DGAKI, ÄDA, GPA und DGG [In-vitro allergy diagnosis, guideline of the DGAKI, ÄDA, GPA und DGG], Allergo Journal 2010; 19: 110 – 128.

18. Date of information

These instructions for use are valid from 10.12.2015.

19. Ordering information

	Article number
Allergozyme® Total IgE	REF 36040000

20. Manufacturer

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