For in-vitro use only! Instructions for use Gold Standard Diagnostics CD Kassel GmbH

Spec. IgE XL

CE REF 36080000

Enzyme immunoassay for the quantitative determination of allergen-specific IgE in human serum or plasma. Micro-titration plate version for 576 tests

1. Intended use

Enzyme immunoassay for the quantitative determination of allergen-specific IgE in human serum or plasma. Determination of specific IgE with this test kit is validated in association with the Gold Standard Diagnostics CD Kassel test system and the determined performance data have been established for the Gold Standard Diagnostics CD Kassel 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. IgE which is bound to the surface of mastocytes and/or 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. Cell-bound specific IgE cannot be determined with the test procedure for the detection of circulating IgE. Therefore, the results should only form part of a diagnostic concept for the determination of the specific IgE in serum, which also includes a detailed history and skin and challenge tests.

3. Test Principle

The quantitative determination of the circulating specific IgE in serum is carried out by means of a non-competitive enzyme immunoassay. The solid phase consists of a chemically activated paper disc on which the corresponding allergen covalents are bound. In the first step, the patient's serum or plasma is pipetted onto the allergen disc. Here, the allergen-specific IgE binds with the allergen which is bound in the solid phase. Excess serum or plasma is then removed in a washing stage. In the second step an enzyme-labelled anti-human-IgE is placed on the disc which contains the allergen-IgE complex. Here the marked anti-human IgE is bound to the specific IgE which is bound to the solid phase. Unbound anti-human-IgE is removed in a washing stage. The quantity of bound and marked anti-human-IgE is proportional to the quantity of the specific IgE in the 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 calibration curve consisting of the extinction values of the measured calibration wells.

4. Content of the specific IgE XL test kit

1. CONJ Conjugate: 1 bottle with 35 ml monoclonal anti-human-IgE (mouse), conjugated with alkaline phosphatase in a buffered protein solution; preservation agent: 0.02% sodium azide, green colored.
2. WASH 20x Washing solution (concentrate): 3 bottles with 60 ml concentrated sodium chloride solution with Tween 20; preservation agent: 0.05% sodium azide (for preparation of the washing solution see 10.2).
3. SUBS Substrate: 1 bottle with 85 ml p-nitrophenyl phosphate (pNPP).
4. STOP Stop solution: 1 bottle with 85 ml 1M sodium hydroxide solution.
5. Calibration system:
   CAL DISC *Calibration discs:* 3 x 10 calibration discs (anti-human-IgE), preservation agent: 0.02% sodium azide.
   CAL SERUM *Calibration serums 1, 2, 3, 4, 5:* 5 bottles, each with 0.6 ml human serum with total IgE calibrated against WHO IRP 11/234. Preservation agent: 0.02% sodium azide. Contains bovine serum albumin (BSA). The calibrators are filled in increasing concentrations:
   CAL SERUM 1 Calibrator 1 = 0.35 IU/ml;
   CAL SERUM 2 Calibrator 2 = 1.0 IU/ml;
   CAL SERUM 3 Calibrator 3 = 3.5 IU/ml;
   CAL SERUM 4 Calibrator 4 = 10 IU/ml;
   CAL SERUM 5 Calibrator 5 = 50 IU/ml.

5. Additional materials and devices

1. Allergen discs:

   Allergen discs are available in packages containing 10 or 25 discs. Preservation agent: 0.02% sodium azide.

2. Materials and equipment:
   - Microtiter plates
   - Disposable gloves
   - Tweezers
   - Distilled water
   - *Gold Standard Diagnostics CD Kassel -System*

6. Limitations of the procedure

- Reliable and reproducible results can only be obtained if the test is performed correctly (see test procedure, Section 10).
- The use of samples other than human serum or plasma has not been validated in this test.
- There is no reuse protocol for this product.
- The clinical diagnosis should not simply be based on the sole evidence of specific antibodies, but rather on other clinical data and test results. The in-vitro determination of specific IgE should never be used as the sole diagnostic decision criteria for starting any hypersensitisation treatment. In addition, skin tests and – if possible - challenge tests should be performed to provide evidence of clinical relevance.
- Especially in the case of allergies to foodstuffs, there may be a negative in-vitro result although there are severe clinical symptoms. This can be explained by the fact that foodstuffs undergo significant changes due to maturing, industrial processing, boiling or frying etc., as well as due to the digestion process, so that under certain circumstances protein structures completely different from those on the solid phase of the allergen substrate may be present. Furthermore, several foodstuffs are highly sensitive, so that not all of the allergens which are present in the native state can be bound to the solid phase.
- Human serum albumin is used as a spacer substance for the in-vitro determination of haptenes. With this, a reproducible pseudo-antigen for in-vitro determination is obtained. Of course, this process cannot completely depict the possible reactions of a haptene in the human body. Because of this, the in-vitro test cannot produce a positive result in all cases where there are positive clinical symptoms.
- In general, negative values for insect toxins only provide evidence that at the time, no circulating specific IgE against the tested insect toxins can be detected in serum or plasma. This does not lead to the conclusion that the patient will not at present, or in the future, develop clinical symptoms in case of an insect sting. In the case of insect toxins, there may be a temporary consumption of the antibodies some time after exposure, so that no specific IgE antibody titre can be detected at the time of the measurement.
- Negative in-vitro 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 specific IgE against the antigen;
  - 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 specific IgE in-vitro test need not automatically cause the same clinical symptoms.
  Many IgE antibodies show a cross-reaction with other IgE antibodies, e.g. birch pollen/apple, mugwort pollen/celery, latex/banana. The diagnosis must take this into account.

7. Specific performance data

Parallelism (representatively for trueness)
For representative allergens from 6 allergen groups a mean inter-dilution coefficient of variation of 23.5 % (basis: units) was determined with 3 samples and 4 consecutive dilution levels each. However, deviating results can be found due to varying composition of the human sample material.

Precision
Repeatability (Intra-Assay):

Sample	Mean (EKL)	CV [%] (basis: classes)	Mean [U/ ml]	CV [%] (basis: units)
1 (n=10)	2.7	2.7	2.8	6.7
2 (n=10)	3.2	1.7	7.4	9.6
3 (n=10)	4.2	4.4	24.9	23.4

Reproducibility (Inter-Assay):

Sample	Mean (EKL)	CV [%] (basis: classes)	Mean [U/ ml]	CV [%] (basis: Units)
1 (n=10)	2.6	5.4	2.7	19.5
2 (n=10)	3.2	1.5	6.9	13
3 (n=10)	4.0	4.7	20.6	23.8

Analytical sensitivity
Slope of calibration curve: The slope between the calibration points is at least

CAL 1 to 2: ≥ 0.11
CAL 2 to 3: ≥ 0.13
CAL 3 to 4: ≥ 0.08
CAL 4 to 5: ≥ 0.01

Measurement range
0.35-50 U/ml
Lowest detection level
< 0.35 U/ml
Metrological traceability of calibrators
WHO IRP 11/234
Analytical specificity
The test is not affected by IgE of other specificity also present in the sample, unless cross-reactivities exist among the allergens.

Further performance data can be provided by Gold Standard Diagnostics CD Kassel upon request.

8. Relevant interferences

Bilirubin conjugated Bilirubin unconjugated	< 0.05 mg/ml < 0.15 mg/ml	no impairment
Haemoglobin	< 5 mg/ml	
Triglyceride	< 5 mg/ml	
Non-specific IgE	< 700 U/ml	

However, deviating results can be found due to varying composition of the human sample material.

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

The reagents are designed for a test run with 576 determinations. Mixing of several test kits with the same Lotnumber is possible.

1. Before starting the test, all components must be brought to room temperature (RT, 20 to 25 °C).
2. Preparation of the washing solution: for 2 plates take 60 ml of the washing solution concentrate to 1.200 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. All reagents and samples must be thoroughly mixed before charging the *Test-System*. Remove the stoppers from the reagent bottles and the serum vials.
4. Start the *Gold Standard Diagnostics CD Kassel-System*. Follow the instructions in the manual or the interactive software of the Test-System.
5. Once the test has been started it must be continued without interruption, and all individual steps, temperatures and reaction times must be complied with.
6. For each test run, values for quality 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 values out of specifications, significant 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 Gold Standard Diagnostics CD Kassel.

Manual test procedure

1. Before starting the test, all components must be brought to room temperature (RT, 20 to 25 °C).
2. Preparation of the washing solution: dilute 60 ml of the washing solution concentrate to 1.2000 ml with distilled water. After dilution the solution can be stored for 24 hours at room temperature if thoroughly cleaned vessels are used.
3. All reagents and samples must be thoroughly mixed before starting the test.
4. Create a distribution scheme for calibrators and examination samples.
   Please note: A double determination of the calibration values is necessary.
5. With a pair of tweezers, place the calibration discs and the allergen discs with the specific allergens in the wells provided. Well A1 (substrate blank value) remains empty. (Recommendation: first place the allergen discs and then the calibration discs)
6. Pipette 50 µl of the each of the calibration serums 1 – 5 onto the corresponding calibration discs and 50 µl of the serum or plasma samples into the wells provided. Well A1 (substrate blank value) remains empty.
7. Cover the microtiter plate and incubate for 1 hour at 37 °C.
8. Wash the wells of the microtiter plates either with the automatic washer or with the manual washer. (Wash volume 300 µl, wash cycles 5, soak time 80 sec.) Only washing procedures approved by Gold Standard Diagnostics CD Kassel must be used.
9. Pipette 50 µl of the green conjugate solution directly onto each of the discs, however not into the blank substrate value. Then cover the microtiter plate again. Incubate for 1.5 hours at 37 °C.
10. Wash as described under 10.8.
11. Pipette 100 µl substrate solution into all of the wells (including the blank substrate value). Cover the microtiter plate and with exclusion of light incubate for 1 hour at 37 °C.
12. In the same manner and sequence as for pipetting of the substrate solution, now add 100 µl of stop solution to all of the wells (incl. the blank substrate value).
13. After stopping the reaction with the stop solution, the colour complex must be measured within 30 minutes. Place the microtiter plates with the stopped coloured solution in the photometer. The measurement is made through the disc on the base of the microtiter well.

For Gold Standard Diagnostics CD Kassel device systems <u>with</u> Allervance software:
The measurement is made with a 3-wavelength method (405, 450 nm as measurement wavelengths and 620 nm as the reference wavelength). This enables the calculation of the values over a larger measurement range.

For Gold Standard Diagnostics CD Kassel device systems <u>without</u> Allervance software:
The measurement is made at 405 nm and the reference wavelength 620 nm. The combined measurement with 405/620 nm must be adhered to. If the evaluation of the 5th calibrator is not calculated, i.e. the value is not printed out, the measurement range of the photometer has been exceeded. In this rare case, over-pipetting must be carried out. To do this, transfer 250 µl from each well into an empty microtiter plate (same scheme!) and measure again at 405/620 nm.

11. Calculation

With Gold Standard Diagnostics CD Kassel devices calculation of the calibration curve and the evaluation of the measurement results are carried out automatically.

Calibrators	Calibration system (5 calibrators)
1	0,35 IU/ml
2	1,0 IU/ml
3	3,5 IU/ml
4	10,0 IU/ml
5	50,0 IU/ml

The calibration curve can be calculated manually by entering the extinctions determined for the calibrators against the calibration unit values on semi-logarithmic graph paper and connecting the individual points with a ruler. This calibration curve is used to determine the values of the serum or plasma samples.
The following relationship exists between U/ml and allergosorbent test (EAST) classes:

< 0.35 U/ml	= EAST class 0
≥ 0.35 < 0.7 U/ml	= EAST class 1
≥ 0.7 < 3.5 U/ml	= EAST class 2
≥ 3.5 < 17.5 U/ml	= EAST class 3
≥ 17.5 < 50 U/ml	= EAST class 4
≥ 50 U/ml	= EAST class 5

12. Normal values

- Values< 0.35 U/ml = EAST class 0 are considered as negative
- Values ≥ 0.35 U/ml = EAST class ≥ 1 are considered as positive

See also Section 6 "Limitations of the procedure" and references 1 and 2

13. Warnings and precautions

The following rules must be observed:

1. The relevant safety regulations must be observed when handling the test components.
2. Calibrators 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. Do not use damaged or contaminated kit components.
5. Smoking, eating and drinking are prohibited in the laboratory. Do not ingest!
6. Do not suck the pipette with your mouth!
7. Avoid cross-contamination when pipetting!
8. Test components from different batches must not be mixed.
9. Reagents must not be used after the expiry date.
10. Calibrator samples and kit controls must be included with every assay array performed to ensure correct results.
11. The functionality and accuracy of the systems used must be checked regularly. Observe the manufacturer's instructions!
12. Any serious incident that has occurred in relation to this product should be reported to the manufacturer and the regulatory authority in the country where the user and/or patients is established.
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:
    - <u>Conjugate</u> (sodium azide <0.1% w/w CAS 26628-22-8; bovine serum albumin CAS 90604-29-8)
    - <u>Washing solution</u> (sodium azide <0.1% w/w CAS 26628-22-8)
    - <u>Substrate</u> (p-Nitrophenyl phosphate CAS 4264-83-9)
    - <u>Stop solution</u> (sodium hydroxide 1M CAS 1310-73-2)
    - <u>Calibration serums</u> (sodium azide <0.1% w/w CAS 26628-22-8; bovine serum albumin CAS 90604-29-8)

14. Quality control

- *Internal quality control*
  It is recommended that for each test run at least one positive control serum is used like a patient serum are used in the test. Gold Standard Diagnostics CD Kassel provides such control serums. For the positive control, the normal ranges are stated by Gold Standard Diagnostics CD Kassel. If the positive control is within the normal ranges, it can be assumed that the test method is functioning correctly.
  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 sent to the laboratory participating in the external quality control 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 Gold Standard Diagnostics CD Kassel 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 on kit and components. Expiry date is the last day of the month stated 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.

18. Date of information

01.10.2022

19. Ordering information

	Article number
Spec. IgE XL	REF 36080000
Microtiter plates (Greiner)	REF 36921000
Allergen discs	see Gold Standard Diagnostics CD Kassel Allergen disc catalogue

20. Distributor/Manufacturer

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