# abia IgE total

REF DK.048.01.3

IVD

Note: Changes highlighted ★

abia

## Intended use

abia IgE total enzyme immunoassay for the quantitative determination of total immunoglobulin E (IgE) concentration in human serum.

For professional use only.

## Clinical value

IgE is the class of immunoglobulins that are normally found in insignificant amounts in serum and secretions (less than 0.001 % of all serum immunoglobulins). The level of total IgE in the newborn is less than 1 IU/ml. IgE levels increase slowly during childhood, reaching adult levels in the second decade of life. In general, elevated IgE levels indicate an increased likelihood of IgE-mediated hypersensitivity, which is responsible for allergic reactions. However, it is important to note that approximately 30 % of patients with atopic symptoms may have normal levels of total IgE; conversely, elevated levels of IgE may be found in a person without allergy.

These substances cause smooth muscle contraction and ultimately lead to allergic conditions such as wheals and rashes, hives, dermatitis, rhinitis, hay fever, asthma and anaphylactic shock. Infants and children with a family history of atopic allergy are at increased risk of developing the disease and are a prime population for screening.

Significant increases can be seen in sensitised individuals, but also in myeloma, pulmonary aspergillosis and in the active stages of parasitic infections.

## Principle of the test

abia IgE total is a one-step immunoassay, based on the sandwich principle.

The assay uses two monoclonal antibodies of high affinity and specificity, capable of binding to ★ two different epitopes on the intact IgE molecule. The first antibody, immobilised on the microtiter plate, captures the antigen to be determined, while the second, enzyme-linked antibody, present in the Conjugate, acts as a detector.

Unbound components are removed by washing. After addition of the solution containing TMB and hydrogen peroxide, the wells with the bound conjugate develop a blue colour which turns yellow when the reaction is stopped with sulphuric acid.

The colour intensity is directly proportional to the concentration of the IgE molecules in the sample and can be read at 450 nm.

## Kit contents

| | S | |
|---|---|---|
| IgE Ab coated plate | 1 | polystyrene plate 12 × breakable 8-well strips coated with monoclonal antibodies to IgE |
| Conjugate | 1 × 18 ml | ready to use; HRP-labeled monoclonal antibodies to IgE; transparent or slightly opalescent pink liquid |
| ★ Calibrator 0 | 1 × 2.0 ml | protein based buffer not containing IgE; pale yellow liquid |
| ★ Calibrator 1 | 1 × 0.5 ml | protein based calibrator containing IgE in concentration approx. 62.5 IU/ml; pale yellow liquid |
| ★ Calibrator 2 | 1 × 0.5 ml | protein based calibrator containing IgE in concentration approx. 125 IU/ml; pale yellow liquid |
| ★ Calibrator 3 | 1 × 0.5 ml | protein based calibrator containing IgE in concentration approx. 250 IU/ml; pale yellow liquid |
| ★ Calibrator 4 | 1 × 0.5 ml | protein based calibrator containing IgE in concentration approx. 500 IU/ml; pale yellow liquid |
| ★ Calibrator 5 | 1 × 0.5 ml | protein based calibrator containing IgE in concentration approx. 1000 IU/ml; pale yellow liquid |
| ★ Control serum | 1 × 0.5 ml | protein based control containing IgE; pale yellow liquid |
| Washing solution (concentrated 25-fold) | 1 × 50 ml | phosphate saline buffer; colourless or pale yellow liquid |
| ★ TMB/substrate solution | 1 × 12 ml | ready to use; citric acid buffer containing TMB and $H_2O_2$; colourless to pale blue liquid |
| Stopping reagent 0.2M $H_2SO_4$ | 1 × 25 ml | ready to use; 0.20 mol/l sulphuric acid solution; colourless liquid |
| Protective film | 1 | |
| Plastic dish | 2 | |
| Plastic zip-lock bag | 1 | |

★ The calibrators have been calibrated using the WHO 3rd International Standard IgE, NIBSC code 11/234. Exact concentrations of calibrators and control serum are indicated on the labels for each lot. For conventional units: IU/ml x 2.4 = mg/ml.

All components are stable until the expiry date of the kit when stored at 2 - 8 °C in a tightly sealed container. The expiry date is printed on the package.
Once opened, the components should be used within 2 months. Concentration of preserving agents is ≤ 0.1 %.

## Materials and equipment required but not provided

- purified water
- automatic or semiautomatic, adjustable or preset pipettes or multipipettes
- disposable pipette tips
- microplate shaker
- automatic microplate washer
- microplate reader equipped with 450 nm filter

## Safety notes

- The human material used in the preparation of the calibrators and control serum has been tested and found to be negative for hepatitis B surface antigen (HBsAg), HIV-1 p24 antigen, hepatitis C virus antibodies, and human immunodeficiency virus antibodies (HIV-1 and HIV-2).
- Since no known test method can provide complete assurance that infectious agents are absent, reagents and specimens should be handled as if they were capable of transmitting infectious disease; any equipment that comes into direct contact with specimens and reagents should be considered contaminated.
- Do not eat, drink, smoke or apply cosmetics in the laboratory.
- Do not pipette by mouth.
- Avoid contact of reagents and specimens with the skin and mucous membranes; wear lab coats and disposable gloves when handling; wash hands thoroughly after use.
- All materials that have come into contact with specimens or reagents, including liquid and solid wastes, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed of in accordance with local regulations.

## Precautions

- Do not use reagents without label or with damaged label/package.
- Do not use expired reagents.
- Do not change the assay procedure; perform all subsequent steps without interruption.
- Do not mix reagents from different lots.
- Do not mix the caps of vials.
- Do not run the EIA test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals.
- Do not let the wells dry once the assay has been started.
- Do not use the same container and tips for different liquid components of the kit and samples.
- Do not reuse the coated plates.
- Do not reuse the removed protective film.
- Do not expose the reagents to excessive heat or sunlight during storage and test procedure.
- Do not freeze the reagents.

## Collection and handling of specimens

- Collect blood samples according to current practice.
- Use serum for testing; performance of the test has not been evaluated with other biological fluids.
- Separate clots or red cells from serum or plasma as soon as possible to avoid haemolysis.
- Do not use sera preserved with sodium azide.
- Do not use contaminated, hyperlipaemic and hyperhaemolysed samples.
- Specimens with hyperproteinaemia and hyperbilirubinaemia have not been specifically tested.
- Samples with observable particulate matter should be clarified by centrifugation prior to testing.
- Suspended fibrin particles or aggregates may cause reactive results.
- Do not heat the samples.
- ★ Specimens may be stored at 2 - 8 °C for 48 hours or frozen at -20 °C for 1 month.
- No more than one freeze-thaw cycle is allowed.

## Procedural notes

- Allow reagents to stabilise at room temperature (18 - 24 °C) for at least 30 minutes before use. ★
- Check the appearance of the reagents.
- Loss of vacuum in the coated plate bag will not affect the performance of the test.
- Check pipettes and other equipment for accuracy and correct operation.
- The washing procedure is a critical step; see the 'Washing Procedure' section for detailed washer settings.
- For a description of the test procedure with automated analysers, see the "Automated analysers" section.

## Washing procedure

Please contact your representative for protocols for recommended washers and procedures.
In general the following protocol is recommended:

- Use flow-through washing with a volume of not less than 300 µl per well.
- Repeat 5 times.
- Do not allow the wells to dry out during the assay procedure.
- Ensure that no liquid remains in the well (use double aspiration in the last step if possible).
- Avoid decanting the plate.
- A residual volume of less than 10 µl is not critical for subsequent steps in the assay procedure.
- If a microplate washer is used, clean the wash head frequently to avoid contamination.

## Preparation of reagents

| Number of strips to be used | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Working washing solution:** mix the reagents thoroughly by inversion<br>**Stability:** 14 days at 18 - 24 °C or 28 days at 2 - 8 °C | | | | | | | | | | | | |
| Washing solution (concentrated 25-fold), ml | 3.0 | 6.0 | 9.0 | 12.0 | 15.0 | 18.0 | 21.0 | 24.0 | 27.0 | 30.0 | 33.0 | 40.0 |
| Purified water, ml | 72.0 | 144.0 | 216.0 | 288.0 | 360.0 | 432.0 | 504.0 | 576.0 | 648.0 | 720.0 | 792.0 | 960.0 |

## Test procedure

abia IgE total is designed for the quantitative determination of the total immunoglobulin E (IgE) concentration in human serum.

| | |
|---|---|
| **1** | Unpack the required number of coated strips. Return the unused strips to the pouch and reseal the foil-lined package in the plastic zip-lock bag. Do not remove the desiccant. |
| **2** | Analyse each calibrator, control serum and sample in duplicate.<br>Add 20 µl of Calibrators 0 - 5 to the appropriate wells.<br>Add 20 µl of Control serum to the appropriate wells.<br>Add 20 µl of the samples to be tested to the remaining wells.<br>The total time should not exceed 10 minutes. |
| **3** | Add 150 µl conjugate to each well. Cover the plate with a protective film. |
| **4** | Incubate for 45 minutes at 37.0 ± 1.0 °C on a microplate shaker (approx. 500 - 800 rpm). |
| **5** | Remove the protective film slowly and carefully to avoid spillage. Aspirate the contents of all wells into a biohazardous waste container (with disinfectant).<br>Add at least 300 µl of Working Washing solution to each well and aspirate. Repeat this procedure 5 times. If possible, use double aspiration in the last step. |
| ★ **6** | Add 100 µl of TMB/substrate solution to all the wells. Allow the plates to stand for 20 minutes at 18 - 24 °C in the dark. |
| **7** | Add 150 µl of Stopping reagent into each well. Gently mix for 5 - 10 seconds. |
| **8** | Read the optical density at 450 nm using a plate reader within 20 minutes after stopping the reaction. |

## Automated analyzers

Validated protocols for automated analysers can be obtained from your representative.

For instruments without an established validated protocol, follow the "Test procedure" section and ensure that all requirements described in the "Precautions" section are met.

All automated analyser protocols must be fully validated before use.

## Calculation and interpretation of the results

### Assay validation

The results of an assay are valid if the following criteria are met for the controls

★ The absorbance (OD) of the blank (Calibrator 0) should be ≤ 0.100 at 450 nm.
The absorbance (OD) of Calibrator 5 should be ≥ 1.300.

★ The absorbance (OD) of the Control serum should be within the specified range (see label).

### Calculation procedure

**1** Calculate the mean optical density of each calibrator duplicate at 450 nm.

**2** Calculate the average optical density of each sample duplicate.

★ **3** Subtract the mean absorbance value of Calibrator 0 (blank) from the mean absorbance values of the calibrators, control and serum samples.

**4** Plot a calibration curve on linear graph paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis.

**5** Read the sample values directly from the calibration curve.
If immunoassay software is being used, a 4-parameter curve is recommended.

If a sample reads greater than 1000 IU/ml, dilute with Calibrator 0 and multiply the result by the dilution factor.

| **Example** | OD 1 | OD 2 | Mean OD - blank | Value, IU/ml | ★ |
|---|---|---|---|---|---|
| Calibrator 0 | 0.054 | 0.054 | 0.000 | 0.000 | |
| Calibrator 1 | 0.351 | 0.348 | 0.296 | 62.50 | |
| Calibrator 2 | 0.612 | 0.628 | 0.566 | 125.00 | |
| Calibrator 3 | 1.074 | 1.104 | 1.035 | 250.00 | |
| Calibrator 4 | 1.796 | 1.865 | 1.777 | 500.00 | |
| Calibrator 5 | 2.606 | 2.541 | 2.520 | 1 000.00 | |
| Control serum | 0.865 | 0.862 | 0.810 | 183.50 | ★ |

These data are for illustrative purposes only and **should not be used** for sample calculations. Each user should generate their own data and standard curve.

## Performance characteristics

### Analytical sensitivity

The analytical sensitivity (limit of detection) was calculated by determining the variability of the Calibrator 0 based on 12 analyses with additional 2 x SD. The imit of detection was set at 2.50 IU/ml.

### Specificity

No cross-reactivity to human IgA, IgG, IgM was observed with this assay.

| **Precision** | Mean value, IU/ml | SD | CV, % |
|---|---|---|---|
| Intra-assay, sample 1 | 183.00 | 7.450 | 4.10 |
| Inter-assay, sample 1 | 178.20 | 12.180 | 6.80 |

### Accuracy

The assay was compared with an enzyme immunoassay as a reference test. The total number of specimens was 266. The values ranged from 0 to 4 469 IU/ml. The least square regression equation and the correlation coefficient were computed for abia IgE total in comparison with the reference method.

The least squares regression analysis was y = 1.015 (x) + 14.615 with a correlation coefficient of 0.97.

| **Expected normal value** | Range, IU/ml | | |
|---|---|---|---|
| Adult allergy-free population | 0.00 | 190.00 | ★ |

Normal value ranges may vary slightly between laboratories. It is strongly recommended that each laboratory establish its own range of expected normal values.

## Limitations of test

- The assay has been validated for the determination of total immunoglobulin E (IgE) in human serum only.
- Results obtained with this assay should never be used as the sole basis for clinical diagnosis. Any laboratory result is only part of a patient's overall clinical picture.
★ - The assay contains reagents to minimise interference from human anti-mouse antibodies (HAMA) and heterophilic antibodies. However, extremely high titers of HAMA or heterophilic antibodies may interfere with the test results.
- The assay has been tested for a high dose hook effect. No hook effect was observed up to an IgE concentration of 4000 IU/ml.

## References


1. Olivieri V., Beccarini I., Gallucci G., Romano T. and Santoro F. Capture assay for specific IgE. An improved quantitative method. J. Immun. Met. 157: 65-72; 1993.
2. Vercelli D. Molecular regulation of the IgE immune response. Clin. Exp. Allergy 25:43-45; 1995.
3. Stern A., van Hage-Hamsten M., Sondell K., Johansson S.G.O. Is allergy screening of blood donors necessary. Vox.Sang. 69:114-119; 1995.
4. Geha R. S Human IgE. J. Clin. Immun. 68:106-111: 1981.
5. Nye L., Marrett T.G., Landon J., White R.J. A detailed investigation of circulation levels of IgE in normal population. Clin. Allergy. 1:13-24; 1975.
6. Homberger H.A., Yuninger J.W. Laboratory testing in the diagnosis and management of allergic diseases. Clin. Lab. 2:351-388: 1983.
7. Wittig H., Bellott J., Filippi I., Royal G. Age-related serum IgE levels in health subjects and in patients with allergic disease. J.Allergy Clin. Immunol. 66:305; 1980.

## Key to symbols used

| | |
|---|---|
| | Manufacturer |
| IVD | For in vitro diagnostic use |
| REF | Catalogue number |
| LOT | Batch code |
| | Do not use if package is damaged |
| | Do not reuse |
| Σ n | Sufficient for [n] tests |
| | Consult Instructions for use |
| | Caution, consult documents |
| ★ | Changes highlighted |
| ★ YYYY-MM-DD | Date of manufacturing |
| YYYY-MM-DD | Expiry date |
| 2°C 8°C | Storage temperature limitation |

## Hazard and precautionary statements for certain kit components


**Stopping reagent**

| | |
|---|---|
| H315 | Causes skin irritation. |
| H319 | Causes serious eye irritation. |
| P264 | Wash hands thoroughly after handling. |
| P280 | Wear protective gloves / protective clothing / eye protection / face protection. |
| P302 + P352 | IF ON SKIN: Wash with plenty of soap and water. |
| P305+<br>P338+<br>P351 | IF IN EYES:<br>Rinse cautiously with water for several minutes<br>Remove contact lenses, if present and easy to do. Continue rinsing. |


**Conjugate, Calibrators 0 - 5, Control serum**

| | |
|---|---|
| H317 | May cause an allergic skin reaction. |
| P261 | Avoid breathing dust / fume / gas / mist / vapours / spray. |
| P280 | Wear protective gloves / protective clothing/ eye protection / face protection. |
| P302 + P352 | IF ON SKIN: Wash with plenty of soap and water. |
| P333 + P313 | If skin irritation or rash occurs: Get medical advice / attention. |

**Attention!**
For complete precautionary statements and detailed information see safety data sheets (SDS).

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