Vitrotest®

Vitrotest Specific-IgE

ELISA test kit for quantitative determination of specific antibodies of IgE class

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Reference souces

Legend

IVD	For in vitro diagnostic use
REF	TK071

Variant of complectation 192-1T

ELISA test for the diagnosis of infectious diseases TS (ТУ.У 24.4 – 36555928 – 001:2011)

«Vitrotest Specific-IgE»

ELISA test kit for quantitative determination of specific antibodies of IgE class

1. Intended use

ELISA test kit «Vitrotest Specific-IgE» is intended for quantitative determination of specific IgE antibodies in human serum or plasma. Used in combination with biotinylated allergens produced by Ramintek IPC.

The test kit may be used both for the ELISA using automatic pipettes and standard equipment and for setting with the open-system immunoenzymatic automated analyzer.

2. Clinical value

According to WHO, at present in 30-40% of the world there are one or more allergic diseases, about 250 thousand people die of asthma only every year. Allergy is increased sensitivity to a substance (allergen) that occurs when the body's repeated contact with this allergen. In Ukraine there is no actual statistics of allergic diseases. However, when focus on the data of domestic and foreign sources, distribution of allergies in our country is 20-30% (not less than 10 million citizens). The rapid growth in the number of allergic diseases associated with increased allergenic load per person. Hypersensibilization can be caused by pollen, foods (cow's milk, fish, eggs, cereals, vegetables and fruit orange or red, seafood), house dust, fur and particles epithelium of animals, molds and yeasts, drugs, synthetic materials, etc.

The most common manifestations of allergy, according to WHO, is allergic rhinitis (10-30% of the population), allergic conjunctivitis, rhinosinusitis, asthma (20%), food allergy (220-520 million people), anaphylaxis, urticaria and angioedema, allergic to poison stinging insects (often fatal) medical and occupational allergy. Children allergic disease ranks first among chronic diseases, with most of them is food allergy (5 to 15% of children).

All of the above manifestations of allergic diseases are allergic reactions of anaphylactic (immediate) type. It is based on reaginic mechanism of tissue damage that occurs normally with antibodies of class IgE, less IgG4 on the surface of mast cells and membranes. Anaphylactic reaction type occurs in 3 phases: 1 - immunological phase in which allergens combine with immunoglobulin E, which are fixed on basophils and mast cells by changing the properties of cell membranes; 2 - pathochemical or biochemical phase - is the degranulation of mast cells and basophils to release a large number of mediators; 3 – pathophysiological phase - the action of mediators on organs and tissues. Clinical manifestations of reactions occur within the first two hours after contact with a specific allergen.

Today methods of diagnosis of allergy in vitro have increasingly popularity; one of them is enzyme immunoassay (ELISA) for the determination of total IgE which allows to determine the content of total IgE and allergen-specific IgE in serum or plasma. This method has several advantages: no contraindications to survey and age restrictions, does not cause additional sensitization and anaphylactic reactions, characterized by high sensitivity and specificity.

Determining the level of specific IgE should be performed for necessity assessment of sensitivity to certain allergen or inconsistency of results of skin tests and anamnesis. Detection of specific IgE is also used in the differential diagnosis of IgE-dependent allergic reactions, especially in the case of food allergies. Often there are cases where the patient has an increased sensitivity to only one allergen, resulting in total IgE levels may be within normal limits, while the skin test and ELISA results for detection of specific IgE are positive.

3. Principle of the test

Principle of the test of «Vitrotest Specific-IgE» kit is based on «capture» solid phase immunoassay technique using biotinylated allergens.

The solid phase is made of strip microplate coated with the first monoclonal antibodies specific to human IgE. Wells of plate are used both for determination of IgE using biotinylated allergens and to construct the calibration graph. Calibrators are added to the wells of first strips and investigated samples - in all other wells. During incubation of calibrators and samples in the wells is going "capture" of IgE by monoclonal antibodies on the solid phase. After washing out unbound components into the wells with calibrators are added monoclonal anti-IgE antibodies, and into the wells with samples – different biotinylated allergens. During incubation in the wells with calibrators has been forming “sandwich”: anti-IgE + IgE + anti-IgE-biotin, and in wells with samples - complex: anti-IgE + IgE + allergen-biotin. After washing out unbound components the conjugate solution of streptavidin with horseradish peroxidase added to the wells, and binds to biotin in the complex formed on solid phase. Unbound components are washed out. The immune complex formed in the wells are visualized by adding chromogen solution of 3,3',5,5'-tetramethylbenzidine (TMB). As a result of the reaction solution in wells where immune complexes were formed would be painted in blue. The reaction is stopped by adding stop reagent, blue colored wells become yellow. The result of the analysis is determined by spectrophotometric reading at 450/620 nm.

Internal calibrators of the test-kit «Vitrotest Specific-IgE» are standardized with The 2d International Standard WHO 75/502, units of measurement are IU/ml.

4. Materials and equipment

4.1 Contents of the kit

ELISA plate – 12 strips of 8 wells (with the possibility of separation of the wells) with immobilized monoclonal antibodies to human IgE.

Calibrator C0 – 1 vial containing 0,7 ml negative serum (yellow).

Calibrator C0,35 – 1 vial containing 0,7 ml serum containing IgE with concentration 0,35 IU/ml (yellow).
Calibrator C1 – 1 vial containing 0,7 ml serum containing IgE with concentration 1 IU/ml (yellow).
Calibrator C5 – 1 vial containing 0,7 ml serum containing IgE with concentration 5 IU/ml (yellow).
Calibrator C25 – 1 vial containing 0,7 ml serum containing IgE with concentration 25 IU/ml (yellow).
Calibrator C100 – 1 vial containing 0,7 ml serum containing IgE with concentration 100 IU/ml (yellow).
Control serum - 1 vial containing 0,7 ml serum containing IgE with concentration 2-3 IU/ml (yellow).
Sample diluent – 1 bottle containing 12 ml buffer with detergent and preservative (violet).
Biotinylated anti-IgE antibodies – 1 containig 10 ml buffer solution of biotinylated monoclonal antibodies to human IgE with stabilizers and preservatives (pink). Ready to use.
Conjugate solution streptavidin-peroxidase – 1 vial containing 22 ml buffer solution of streptavidin conjugated with horseradish peroxidase, with stabilizers and preservatives (green). Ready to use.
TMB Solution – 1 bottle containing 12 ml of TMB solution and hydrogen peroxide, with stabilizers and preservatives (colourless).
Washing solution Tw20 (20x) – 1 bottle containing 50 ml 20-fold concentrated phosphate buffer with Tween-20 (colourless).
Stop-reagent – 1 vial containing 12 ml of 0,5 M sulphuric acid solution (colourless).
Adhesive film – 6 sheets of adhesive film for covering the plates during incubation.
Sera identification plan – 1 sheet of paper for noting the schemes of samples distribution.
Form for calibrating graphic – 2 sheets for constructing calibration graph.
Instruction for use – one copy of user manual.

4.2 Additional reagents, materials and equipment

In order to conduct the analysis, the following additional reagents, materials and equipment are required:
– Biotinylated allergens produced by Ramintek IPC – each vial containing 1,8 ml buffer solution of biotinylated allergens with stabilizers and preservatives (yellow). Ready to use. Biotinylated allergens are listed in the catalogue. The contents of one vial is sufficient for 16 determinations. The labels of bottles specified code numbers and names of allergens.
– deionized or distilled water;
– filter paper;
– graduated cylinders of 10, 200 and 1000 ml capacity;
– disposable gloves;
– hydrogen peroxide solution 6%;
– disposable glassware for preparing the reagents (bottles and trough);
– timer;
– mono- and multi-channel automatic adjustable pipettes capable of delivering volumes of 20, 200 and 1000 microliters and tips for them;
– thermostat for 37 °C;
– container for solid waste;
– container for liquid waste;
– [1]automatic or semi-automatic washer;
– [2]mono or multi-channel reader for microplates at 450/620-695 nm.
[1,2] Please, consult us about the adaptation of your equipment.

5. Reservations and safety precautions

5.1. Reservations:
– do not use expired reagents;
– do not use in the analysis and do not mix components of different lots and components of test kits with different nosology;
– do not use reagents of other manufacturers in composition with the Vitrotest® sets;
- Note: possible to use washing solution Tw20 (20X), TMB solution and Stop-reagent with other series that are different from those attached to the test kit. These reagents are used in other test systems of Ramintek IPC. Please consult us for details.
– close reagent vials after use only with their appropriate caps;
– strictly follow to the washing procedure requirements described in this instruction;
– control the filling and full aspiration of the solution in the wells;
– use a new distribution tip for each serum and reagent;
– protect kit reagents from straight sun rays;
– TMB solution must be colourless or light blue before it's using. If solution is dark blue or yellow, it can't be used. Avoid any contact of the TMB solution with metals or metal's ions. Use glassware thoroughly washed and rinsed with deionized water.
– use only disposable pipette tips for adding TMB-substrate into plate's wells;
– never use the same glassware for conjugate solution and chromogen.

5.2. Safety precautions:
– all reagents included in the kit are intended for "in vitro" diagnostic use;

– wear disposable gloves when perform analysis;

– do not pipette by mouth;

– the calibrators of the kit «Vitrotest Specific-IgE» are nonreactive for HBsAg and antibodies to HIV ½, HCV, *Treponema pallidum*. Nevertheless, all controls and sera should still be regarded and handled as potentially infectious;

– the liquid waste must be inactivated with, for example, the hydrogen peroxide solution at the final concentration of 6% for 3 hours at room temperature, or with the sodium hypochlorite at the final concentration of 5% for 30 minutes, or with other disinfectant agents;

– the solid waste must be inactivated with autoclaving at 121°C for 1 hour;

– do not autoclave the solutions that contain sodium azide or sodium hypochlorite;

– avoid spilling of TMB-solution and Stop-reagent and any contact of these solutions with the skin or mucosa;

– in case of spilling of solutions, that do not contain acid, e.g. sera, rinse the surface with hydrogen 6% solution, then dry with filter paper.

6. Storage and stability

Reagents of the kit are stable up to the expiry date on the label, when stored at 2-8 °C.

Transport the test-kit at 2-8 ºC. Disposable transportation at temperature not higher than 20ºC during two days is allowed.

7. Specimen collection

The serum or plasma samples should be stored at 2-8 ºC not more then 3 days after collection. It is possible to store them longer, but frozen (-20 to-70 ºC). Before use frozen samples, wait for 30 minutes for the reagents to stabilize at room temperature. Mix thawed samples well to homogeneity. Avoid repeated freezing/thawing. Samples containing aggregates must be clarified by centrifugation. Do not use samples with contaminated, hyperlipeamic and hyperhaemolysed sera.

8. Reagent preparation

It is very important to bring all reagents of the «Vitrotest Specific-IgE» kit to room temperature (18-25°C) for 30 minutes before use.

Test kit «Vitrotest Specific-IgE» is used together with biotinylated allergens produced by "Ramintek IPC", so biotinylated allergens should be also prepared. Select allergens required to analyze and keep them at room temperature 18-25 °C for 30 minutes before use.

8.1. ELISA plate preparation

Before opening the ELISA plate, allow it to stabilize at room temperature for 30 minutes to avoid water condensation inside the wells. Open the vacuum bag and remove necessary amount of wells. Immediately after removal of wells, the remaining strips should be **resealed with zip-lock and stored at 2-8 °C**. Microplate in thus packed bag is stable for 3 month.

8.2. Washing solution

The vial contains 50 ml of a concentrated 20X buffer with detergent. Dilute the washing solution 1:20 (1+19) with distilled or deionised water, then mix. For example: for 8 ml of concentrate – 152 ml of distilled water is enough for 4 strips. Crystals in the solution disappear by warming up to 37°C for 15-20 min.

The diluted washing solution can be stored at 2-8°C not more than 7 days.

9. Assay procedure

9.1. Take out from the protective packing the support frame and the necessary number of wells (the number of investigated samples and wells for calibrators). Calibrators are added in wells of the first strips, and in all other wells dispense samples. The number of repetitions of each sample is determined by the number of investigated allergens. Wells with calibrators must be included in each analysis.

9.2. Complete the sera identification plan.

9.3. Prepare washing solution (refer to point 8.2).

9.4. Dispense 50 µl of sample diluent in each well.

9.5. Dispense 50 µl of calibrators and unknown samples into respective wells (see scheme on the last page). Gently pipette the mix in wells, avoiding foaming. After addition of serum color of the solution in wells changes from violet to blue.

9.6. Cover strips with an adhesive film and incubate for 30 min at room temperature 18-25 °C.

9.7. After completing the incubation remove the adhesive film carefully and wash the wells five times using the automatic washer or 8-channel pipette in the following order:

– aspirate the content of wells strips into a liquid waste container;

– fill the strip wells with a minimum of 300 microliters of washing solution for each well (respect the soak time of a minimum of 30 seconds);

– aspirate the solution of all wells, the residual volume of solution after aspiration must be lower than 5 microliters;

– repeat the washing step four more times;

– after the last aspiration blot the microplate by turning it upside down on absorbent paper.

9.8 Dispense 100 µl of biotinylated anti-IgE antibodies into wells with calibrators and 100 µl of each biotinylated allergen into wells with samples (see scheme on the last page).

9.9. Cover strips with an adhesive film, incubate for 30 min at room temperature 18-25°C.

9.10. After completing the incubation remove the adhesive film carefully and wash the wells five times as described above (refer to point 9.7).

9.11. Dispense 100 µl of conjugate solution streptavidin-peroxidase per well.

9.12. Cover strips with an adhesive film, incubate for 15 min at room temperature 18-25°C.

9.13. After completing the incubation remove the adhesive film carefully and wash the wells five times as described above (refer to point 9.7).

9.14. TMB-solution is ready to use TMB-substrate solution with hydrogen peroxide. TMB-solution should be colorless, protect TMB-substrate solution from straight sun rays. To add TMB-solution only new tips must be used: carefully select a TMB-solution from the vial and without touching the bottom and walls of the hole plate, add 100 µl TMB solution per well.

9.15. Incubate the strips for 30 minutes at room temperature of 18-25°C in the dark.

9.16. Add 100 µl of stop-reagent in each well. Respect the same distribution sequence than for the TMB-substrate solution.

9.17. Read the optical density of every strip well in dual wavelength reading at 450/620 nm, within the 5 minutes of stopping the reaction. Pay attention to the cleanness of the wells bottom outside.

Measurement in the single-wave procedure at 450 nm is possible. Reserve blank well to adjust spectrophotometer in such analysis. Only TMB-substrate and stop-reagent must be added in blank well.

10. Calculation and interpretation of the results

10.1. Test validity conditions:

In order for an assay to be considered valid, the following criteria must be met

Results of the analysis considered valid only if the OD of each of calibrators in Table 1 is in the specified range of values and the concentration of control serum is in the range indicated on the label of vial (2- 3 IU/ml).

Table 1

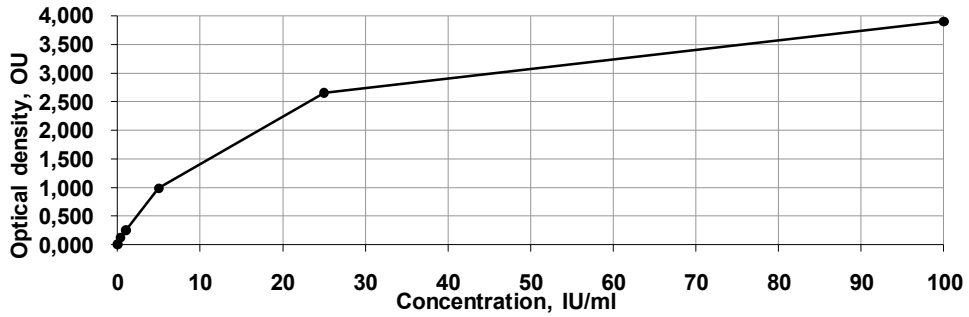
Calibrators	OD value
C0	**Not higher than 0,07 OU**, ie, OD C0≤ 0,07
C0,35	**Not lower than 0,1 OU**, ie, OD C0,35 ≥ 0,1
C1	**Not lower than 0,2 OU**, ie, OD C1 ≥ 0,2
C5	**Not lower than 0,7 OU**, ie, OD C5 ≥ 0,7
C25	**Not lower than 1,0 OU**, ie, OD C25 ≥ 1,0
C100	**Not lower than 1,7 OU**, ie, OD C100 ≥ 1,7

If the data obtained are beyond the specified values, the results should be considered unreliable and analysis should be repeated.

10.2. Calculation of the results.

To obtain quantitative results of determination the concentration of specific IgE antibodies in IU/ml construct a calibration graph: on the axis OY set aside six calibrators OD values C0, C0,35, C1, C5, C25 and C100, and the OX axis set aside corresponding concentrations - 0, 0,35, 1, 5, 25, 100 IU/ml, respectively.

Using the calibration graph determine the concentration (IU/ml) of specific antibodies in the samples, which corresponds to the obtained OD. **Example** of calibration graph shown in Figure.



Note: Do not use this graph to determine the concentration of total IgE antibodies in Your analysis.

Calibration samples standardized with The 2nd WHO International Standard 75/502 for total IgE. **Since there is no international standardization for measurements of specific IgE and reference values for allergens, the concentration of specific IgE obtained by different methods can differ. Accordingly, you should be careful when comparing results from different methods.**

For convenience of calculating results can be used computer programs for reading and calculation results of research.

10.3. Interpretation of the results.

The results of determining the concentration of specific IgE in IU/ml interpreted as follows:

Concentration	*Result*
< 0,35 IU/ml	**Not detected**
0,35-1 IU/ml	**Low level**
1,1-5 IU/ml	**Medium level**
5,1-100 IU/ml	**High level**
>100 IU/ml	**Very high level**

11. Performance characteristics

11.1. Specificity and sensitivity

Monoclonal anti-IgE antibodies used it the test show no cross-reactivity with human immunoglobulin classes A, M, G

To verify specific reactivity of the test «Vitrotest Specific-IgE» was used control serum BIOREF-AL20 containing allergen-specific IgE (BIOREF GmbH, Germany). This model is offered in two clinically relevant concentration ranges of IgE (Level 1 and 2). Content of specific IgE are listed below in the graph "acceptable range".

Allergen ID	Name of allergen	Level 1, IU/ml		Level 2, IU/ml	
		Acceptable range	**Data obtained**	Acceptable range	**Data obtained**
D1	House dust mites *D.pteronyssinus*	0,27-1,28	**0,98**	1,9-9,1	**7,13**
D2	House dust mites *D.farinae*	1,18-5,58	**1,43**	9,8-46,2	**10,9**
E2	Epithelium of cat	0,44-2,06	**1,42**	3,2-15,1	**5,5**
G6	Timothy pollen	0,79-3,74	**1,59**	7,2-33,8	**8,41**
G12	Cereal ruttishness pollen	0,50-2,34	**1,02**	4,4-20,6	**7,96**
F2	Cow's milk	0,41-1,91	**1,62**	3,4-16,0	**3,9**
F13	Peanuts	0,64-3,04	**0,82**	3,6-17	**3,84**

11.2. Accuracy

Intra assay reproducibility

Coefficient of variation (CV) for two sera with specific IgE to different allergens was calculated in 32 repetitions on two series of test kits - CV_1 and CV_2.

Inter assay reproducibility

Coefficient of variation (CV) for two sera with specific IgE to different allergens was calculated for three days in three ELISA performances, in eight repetitions for each analysis - **CV_3**.

The table shows the results obtained for some allergens belonging to different groups:

Allergen ID	Name of allergen	Serum №	CV_1, %	CV_2, %	CV_3, %
D1	House dust mites *D. pteronyssinus*	59	2,9	4,8	6,3
		172	2,1	3,0	2,9
E2	Epithelium of cat	75	3,4	2,8	3,1
		42	1,6	1,8	2,0
T3	Birch pollen	53	3,6	3,1	3,3
		405	2,7	2,3	2,6
W1	Ragweed pollen	13	3,6	4,9	5,6
		17	6,6	6,8	7,2
F1	Chicken egg white	176	2,0	1,9	3,6
		128	1,8	1,7	2,2

12. Limits of the test

Reliable results can be obtained only in full respect of the recommendations of instruction to the test «Vitrotest Specific-IgE». Cannot be used in the analysis the sera samples with severe lipidemia, hemolysis, and bacterial germination.

Past studies of the identification of allergen-specific IgE demonstrated the effectiveness of determination of allergens that caused hypersensibilization for clinical evaluation of allergic conditions. **However, any result of the identification of specific IgE does not guarantee absence or presence of allergic diseases.** The final diagnosis can be established only on the basis of clinical symptoms, medical history and data set of laboratory tests.

Affine constants of specific IgE may be different for different allergens, so you need to keep in mind that identical results for different allergens may not fully reflect the clinical condition.

Values for specific IgE obtained in the test kits from different manufacturers can vary due to differences in methods of analysis and specificity of reagents (especially allergens, depending on the way they receive). That concentration of allergen-specific IgE obtained in different assays may not be equivalent.

It should be noted that the food allergies even when there is a clear clinical picture, specific IgE could not be detected because the reaction can develop both IgE-mediated mechanism and without IgE.

It should also be remembered that IgE, specific to a particular allergen, may exhibit cross-reactions within the group, due to the presence of common antigenic determinants.

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Legend

Interpretation of notation conventions:

IVD	For in vitro diagnostic use
LOT	Batch code
REF	Catalogue number
	Production date
	Expiry date
	Storage temperature limitation
	Meant for <n> tests
	Protect from direct solar radiation
	Attention! Consult instruction for use
	Manufacturer and its address
	Consult instructions for use

For questions and suggestions regarding the kit, contact the manufacturer:

Ramintek Innovation-Production Company
03039 Ukraine, Kiev, 7 October 40th Anniversary Av., of. 227 (registered address)
07300 Vishgorod, Kiev region, 19 Sholudenko Str. (factual address)
Tel. +380 44 222-76-72

Scheme of the assay «Vitrotest Specific-IgE» with use of biotinylated allergens

Keep reagents of the kit «Vitrotest Specific-IgE» and biotinylated allergensat room temperature 18-25°C during 30 minutes

Prepare washing solution, dilute 20x concentrate washing solution *Tw20* with distilled water 1:20 (1+19). For example, 8 ml of solution + 152 ml of water

Complete the sera identification plan

Dispense 50 µl of sample diluent into the wells

Wells for calibrators	***Wells for samples***
Dispense 50 µl of each calibrator	**Dispense 50 µl of each sample**

Color in wells switches from violet to blue

Cover wells with adhesive film, incubate for 30 min at room temperature 18-25°C

Wash wells five times

Wells for calibrators	***Wells for samples***
Dispense 100 µl of biotinylated anti-IgE antibodies	**Dispense 100 µl of biotinylated allergen**

Cover wells with adhesive film, incubate for 30 min at room temperature 18-25°C

Wash wells five times

Dispense 100 µl of conjugate solution streptavidin-peroxidase

Cover wells with adhesive film, incubate for 15 min at room temperature 18-25°C

Wash wells five times

Dispense 100 µl of TMB substrate solution into the wells

Incubate the plate for 30 min in the dark at room temperature (18-25°C)

Add 100 µl of stopping solution in each well

Read optical density at 450/620 nm

Construct a calibration graph, determine the concentration IU/ml for specific IgE antibodies in the samples

Interpret the results according to the table

Concentration	*Result*
< 0,35 IU/ml	**Not detected**
0,35-1 IU/ml	**Low level**
1,1-5 IU/ml	**Medium level**
5,1-100 IU/ml	**High level**
>100 IU/ml	**Very high level**