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User's Manual

IgG Screen Nutritional ELISA Kit

REF **DEIA301**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The IgG Screen Nutritional 24 ELISA test kit has been designed for the detection and the quantitative determination of specific food antigen-related IgG antibodies in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service. This assay is intended for research use only.

General Description

Incompatibility reactions against food may cause various symptoms in the human organism and this disturbance is manifested in the immune system by the formation of specific IgE as well as IgG and/or IgG4 antibodies. Statistics show that 60% of the population suffer from intolerances against at least one foodstuff, which may cause clinical symptoms or enhance them. Hints may be various and reach from skin irritations over digestive disorders up to migraine. With the diagnostic findings of unspecific discomfort, allergies or intolerances against food should be clarified. The theoretical basis for the determination of specific IgG or IgG4 for the diagnosis of food intolerances depends on the observation that some subclasses of IgG (mainly IgG4) are connected to the in vitro degranulation of basophilic cells and mastocytes and the activation of the complement cascade. It was also observed, that high concentrations of circulating IgG were measured in atopic persons. Already early surveys showed that in persons with inflammatory reactions against food IgG but not IgE was detected. Significantly enhanced IgG and IgG4 titers were also found in patients with food intolerances. Skin tests are relatively poorly correlated to food allergies and are only significant in the presence of IgE related reactions. As additional diagnostic tools provocation and elimination diets are applied. These methods depend strongly on the motivation and compliance of the patient. Due to these constraints nowadays serological determinations of antibodies against various food panels are applied increasingly. The two reactions related with the immune system differ insofar as the IgE associated food allergy occurs within the next hour following the food intake, while IgG/IgG4 intolerances show a delayed reaction of 24 to 120 hours and persistent symptoms may arise.

Principles of Testing

The IgG Screen Nutritional 24 ELISA test kit is based on the principle of the enzyme immunoassay (EIA). Three samples can be tested with each kit. Four color-coded strips are required per sample. 24 different food antigens and 8× reference antigen (egg white) for standards and controls are bound to these four strips. Diluted serum or ready-touse standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized antigens takes place. After one hour incubation at 37°C, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG-AP conjugate is added and incubated for 30 minutes at 37°C. After a further washing step, the substrate (PNPP) solution is pipetted and incubated for 60 minutes at 37°C, inducing the development of a yellow dye in the wells. The colour development is terminated by the addition of a stop solution. The resulting dye is measured spectrophotometrically at the wavelength of 405 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the colour.

Reagents And Materials Provided

The IgG Screen Nutritional 24 ELISA kit contains one microtiter plate and sufficient reagents for the testing of 3 samples (24 determinations per sample) as well as the generation of 3 standard curves and the testing of controls. The strips and solutions have to be stored at 2-8°C. The expiry date is mentioned on the labels.

Microtiter Strips: For 3 samples with 4 color coded strips with 8 wells each. There are 4 different strips (coded with violet, green, yellow and red color) required per screen (sample). 8× reference antigen is bound on the violet strip. 24 (3×8) food antigens are bound on the green, yellow and red strips. See attached distribution scheme. Ready to use.

Standards: 6×0.5 mL, human plasma diluted with PBS/BSA, with 0.35, 0.7, 3.5, 17.5, 50 and 100 U/mL of IgG antibodies to egg white. Addition of 0.05% sodium azide. Ready to use.

Low Positive Control: 1 x 0.5 mL, human plasma including low concentrations of IgG antibodies. Addition of 0.05% sodium azide.

High Positive Control: 1 x 0.5 mL, human plasma including high concentrations of IgG antibodies. Addition of 0.05% sodium azide.

Sample Diluent: 40 mL, Tris/BSA buffer. Addition of 0.05% sodium azide. Ready to use.

Conjugate: 15 mL, mouse-a-human-IgG-AP, in proteinacious buffer solution. Ready to use.

Substrate: 15 mL, Paranitrophenylphosphate (PNPP), Ready to use.

Stop Solution: 15 mL, 1 M sodium hydroxide. Ready to use.

Washing Buffer: 60 mL, PBS + Tween 20, 10× concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

Plastic Foils: 2 pieces to cover the microtiter strips during the incubation.

Instruction Booklet

Distribution Scheme

Materials Required But Not Supplied

1. 100 µL and 1000 µL micropipets and multichannel pipets
2. Microtiter plate photometer (405 nm)
3. Microtiter plate washer
4. Tubes for the serum dilution
5. Double-distilled water

Storage

Store kit at 2-8°C. It is stable up to the expiry date stated on the label of the box. Do not use kit beyond its expiry date.

Specimen Collection And Preparation

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the

blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (4-8°C) for up to 48 hours, for a longer storage they should be kept at -20°C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results. For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 30 µL serum + 3 mL sample diluent). Thus for the 24 tests per sample screen only 30 µL serum is necessary.

Reagent Preparation

Washing Solution: dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

Assay Procedure

General Remarks:

1. Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
2. All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
3. Return the unused microtiter strips to the plastic bag and store them dry at 4-8°C.

Procedure:

1. For each sample prepare one set of four one microtiter strips.
2. Pipet 100 µL each of the diluted (1:101) sample and the ready-to-use standards or controls respectively into the wells according to the distribution scheme.
3. Cover plate with the enclosed foil and incubate at 37°C for 60 minutes.
4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
5. Pipet 100 µL each of ready-to-use conjugate into the wells.
6. Cover plate with the enclosed foil and incubate at 37°C for 30 minutes.
7. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
8. Pipet 100 µL each of the ready-to-use substrate into the wells.
9. Cover plate with the enclosed foil and incubate at 37°C for 60 minutes in the dark (e.g. drawer).
10. To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 405 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

Evaluation

The evaluation can be performed either in units per mL (U/mL) or in classes.

Quantitative Evaluation

The ready-to-use standards of the IgG Screen test kit are defined and expressed in arbitrary units (U/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given sample follow-up controls become possible.

For a quantitative evaluation the absorptions of the standards and controls are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs.

Performance Characteristics

Spec. IgG ELISA	Egg white	Cow milk	Tomato
Intra-Assay-Precision	10.9 %	9.2%	7.9%
Inter-Assay-Precision	8.0 – 11.6 %	15.5 – 17.4 %	5.7 – 11.6 %
Inter-Lot-Precision	1.2 – 8.0 %	3.0 – 19.0 %	7.2 – 9.8 %
Analytical Sensitivity	0.18 U/mL	0.10 U/mL	0.11 U/mL
Recovery	101 – 109 %	89 – 102 %	90 – 97 %
Linearity	85 – 102 %	69 – 93 %	83 – 105 %

Specificity

No cross reactivity towards IgE up to 100.000 IU/mL.

Interferences

No interferences with bilirubin up to 0.3 mg/ mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL

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