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User's Manual

IgG4 Screen Nutritional 20 ELISA Kit

REF **DEIA303**

Σ **96T**

RUO

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 IgG4 Screen Nutritional 20 ELISA test kit has been designed for the detection and the quantitative determination of specific food antigen-related IgG4 antibodies in serum and plasma.

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. Already early surveys showed that in persons with inflammatory reactions against food IgG but not IgE was detected. Significantly enhanced IgG and IgG4 titerswere also found in patients with food intolerances. Skin tests are relatively poorly correlated tofood 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 compliancy of the patient.

Principles of Testing

The IgG4 Screen Nutritional 20 ELISA test kit is based on the principle of the enzyme immunoassay (EIA). Twenty (20) different antigens and 4× reference allergen (egg white,f01) are bound on the surface of the microtiter strips. Diluted serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG4 antibodies of the serum and the immobilized antiges takes place. After a 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-IgG4-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 IgG4 antibodies is directly proportional to the intensity of the colour.

Reagents And Materials Provided

Microtiter Strips: 1 microtiter plate a 96 wells for 4 samples. Three color-coded microtiter strips (green, yellow, red) with 8 wells each, coated with 20 food antigens and 4× reference antigen.

Standards: 4×0.5 mL, human plasma diluted with PBS/BSA, with 0.35, 0.7, 3.5, 17.5U/mL of IgG4 antibodies to egg white(f01). Addition of 0.05% sodium azide. Ready to use.

Anti-human-IgG4 Enzyme Conjugate: 15mL, mouse-a-human-IgG4-AP, in proteinacious buffer solution. Addition of 0.01% methylisothiazolone, 0.01% bromonitrodiozane and 5mg/L Proclin. Ready to use.

Sample Diluent: 40 mL, PBS/BSA buffer. Addition of 0.05% sodium azide. 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.

Materials Required But Not Supplied

1. 30 µL, 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).

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. A standard curve should be established with each assay.
4. Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

Procedure:

1. For each sample prepare three microtiter strips (order: green, yellow, red).
2. Pipet 100 µL each of the diluted (1:101) sample and the ready-to-use standards or controls respectively into

the wells.

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.

A	0.35 U/mL standard	rye flour	carrot	0.35 U/mL standard	rye flour	carrot	0.35 U/mL standard	rye flour	carrot	0.35 U/mL standard	rye flour	carrot
B	0.70 U/mL standard	barley flour	tomato	0.70 U/mL standard	barley flour	tomato	0.70 U/mL standard	barley flour	tomato	0.70 U/mL standard	barley flour	tomato
C	3.5 U/mL standard	orange	hazelnut	3.5 U/mL standard	orange	hazelnut	3.5 U/mL standard	orange	hazelnut	3.5 U/mL standard	orange	hazelnut
D	17.5 U/mL standard	banana	peanut	17.5 U/mL standard	banana	peanut	17.5 U/mL standard	banana	peanut	17.5 U/mL standard	banana	peanut
E	egg white	kiwi	curry	egg white	kiwi	curry	egg white	kiwi	curry	egg white	kiwi	curry
F	cow's milk	strawberry	pepper	cow's milk	strawberry	pepper	cow's milk	strawberry	pepper	cow's milk	strawberry	pepper
G	cod	celery	sesame	cod	celery	sesame	cod	celery	sesame	cod	celery	sesame
H	wheat flour	soy bean	pork (meat)	wheat flour	soy bean	pork (meat)	wheat flour	soy bean	pork (meat)	wheat flour	soy bean	pork (meat)

Evaluation

The ready-to-use standards of the IgG4 Screen Nutritional 20 ELISA test kit are defined and expressed in arbitrary units (U/ mL). This results in an exact and reproducible quantitative evaluation. This values for controls and standards in units are printed on the labels of the vials. For a quantitative evaluation the absorptions of the standards are graphically drawn point-to-point against their concentrations. From the resulting reference curve the concentration values or the respective reaction class for controls and each sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs. As curve fit point-to-point has to be chosen.

Performance Characteristics

Spec. IgG4 ELISA	Egg white	Cow milk	Tomato
Intra-Assay-Precision	7.7 %	8.0 %	8.7 %
Inter-Assay-Precision	6.6-10.9 %	8.4-13.0 %	4.6-7.4 %
Inter-Lot-Precision	2.5-11.4 %	5.6-11.8 %	0.5-9.6 %
Analytical Sensitivity	0.22 U/mL	0.17 U/mL	0.16 U/mL
Recovery	90-107 %	89-103 %	87-97 %
Linearity	82-114 %	73-100 %	102-120 %

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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