



## **IgG<sub>4</sub> Screen Nutritional 24 ELISA**

**Enzyme Immunoassay** based on Microtiter Strips  
for the Detection and Quantitative Determination  
of Human IgG<sub>4</sub> Antibodies against  
**24 Food Antigens**  
in Serum and Plasma

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Cat.-No.: ILE-SCG210  
Storage: 2-8°C  
For in-vitro diagnostic use only

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## **Intended Use**

The Immunolab IgG<sub>4</sub> Screen Nutritional 24 ELISA test kit has been designed for the detection and the quantitative determination of specific food antigen-related IgG<sub>4</sub> antibodies in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of Immunolab.

This assay is intended for in-vitro diagnostic use only.

Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

## **General Information**

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 IgG<sub>4</sub> 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 IgG<sub>4</sub> for the diagnosis of food intolerances depends on the observation that some subclasses of IgG (mainly IgG<sub>4</sub>) 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 IgG<sub>4</sub> 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/IgG<sub>4</sub> intolerances show a delayed reaction of 24 to 120 hours and persistent symptoms may arise.

## Principle of the Test

The Immunolab IgG<sub>4</sub> Screen Nutritional 24 ELISA test kit is based on the principle of the enzyme immunoassay (EIA). Three patients can be tested with each kit. Four color-coded strips are required per patient. 24 different food antigens and 8x reference antigen (egg white) for standards and controls are bound to these four strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG<sub>4</sub> antibodies of the serum and the immobilized food 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<sub>4</sub>-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<sub>4</sub> antibodies is directly proportional to the intensity of the colour.

## Limitations and Precautions

- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking, smoking and application of cosmetics in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- No reagents from different kit lots have to be used, and they should not be mixed with one another.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.

- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and danger of intoxication exists.

## Reagents

The IgG<sub>4</sub> Screen Nutritional 24 ELISA kit contains one microtiter plate and sufficient reagents for the testing of 3 patients (24 determinations per patient) 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 patients with 4 color coded strips with 8 wells each. There are 4 different strips (coded with black, green, yellow and red color) required per screen (patient). 8x reference antigen is bound on the black strip. 24 (3x 8) food antigens are bound on the green, yellow and red strips. See attached distribution scheme. Ready-to-use.

**Standards:** 6x 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<sub>4</sub> 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<sub>4</sub> antibodies. Addition of 0.05% sodium azide.

**High Positive Control:** 1 x 0.5 mL, human plasma including high concentrations of IgG<sub>4</sub> 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<sub>4</sub>-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, 10x 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

## Preparation of Reagents

**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.

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

## Materials Required but not Provided

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

## Specimen Collection and Handling

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 patient screen only 30 µL serum are necessary.

## Assay Procedure

1. For each patient 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  $\mu$ L 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  $\mu$ L 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.

### Example

	<u>Class</u>	<u>Values (OD)</u>
Standard 0.35 U/mL	1	0.156
Standard 0.70 U/mL	2	0.212
Standard 3.50 U/mL	3	0.445
Standard 17.5 U/mL	4	1.035
Standard 50 U/mL	5	1.660
Standard 100 U/mL	6	2.569

The above table contains only an example, which was achieved under in-house temperature and environmental conditions. The described data constitute consequently **no reference values** which have to be found in other laboratories in the same way.

### Quantitative Evaluation

The ready-to-use standards of the IgG<sub>4</sub> 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 patient 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 patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs.

## Assay Performance

Spec. IgG <sub>4</sub> 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 %
Cross reactivity	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		
Clinical specificity	88 %	86 %	90 %
Clinical sensitivity	86 %	94 %	80 %

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### Short Instruction

