Human IgG4 Screen Nutritional 88 ELISA Kit

Enzyme Immunoassay for the quantitative determination of human IgG4 antibodies against 88 food antigens in serum and plasma

Catalog number: ARG80822

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

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, IgG 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.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. 88 different food antigens and 8x reference antigens (egg white) for standards and controls are bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards and controls are pipetted into the wells of the microtiter plate. A binding between the IgG4 antibodies of the serum and the immobilized antigens 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 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 color development is terminated by the addition of a stop solution. The resulting dye is measured at the wavelength of 405 nm. The concentration of the IgG4 antibodies is directly proportional to the intensity of the color.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
88 different nutritional antigens-coated microplate	12 strips x 8-well	4°C
Standards (0.35, 0.7, 3.5, 17.5, 50, 100 U/ml)	6 X 0.5 ml (ready to use)	4°C
Low positive Control	0.5 ml (ready to use)	4°C
High positive Control	0.5 ml (ready to use)	4°C
Sample dilution buffer	40 ml (ready to use)	4°C
AP-antibody conjugate	15 ml	
10x Wash Buffer	60 ml	4°C
PNPP substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.

- Briefly spin down the AP-Antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer and Sample diluent buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

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 (2-8°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. 100 µl serum + 10 ml sample diluent). Thus for the 88 tests per patient screen only 100 µl serum is necessary.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards and samples in duplicate into wells.
3. Incubate for 60 minutes at 37°C.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X wash buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µl of AP-Antibody Conjugate into each well. Incubate for 30 minutes at 37°C.
6. Aspirate and wash well as step 4.
7. Add 100 µl of PNPP substrate to each well. Incubate for 60 minutes at room temperature in dark.
8. Add 100 µl of Stop Solution to each well.
9. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

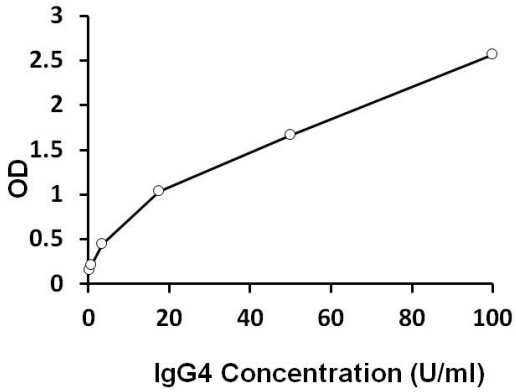
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Spec. IgG4 ELISA	Egg white	Cow milk	Tomato
Analytical sensitivity	0.22 U/mL	0.17 U/mL	0.16 U/mL
Cross reactivity	No cross reactivity towards IgE up to 100.000 IU/mL.		
Intra-assay precision	7.7 %	8.0 %	8.7 %
Inter-assay precision	6.6 – 10.9 %	8.4 – 13.0 %	4.6 – 7.4 %
Recovery	90 – 107 %	89 – 103 %	87 – 97 %
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		