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User's Manual

Human IgG4 Screen Nutritional 88 ELISA Kit

REF **DEIA304**

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.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Enzyme Immunoassay for the quantitative determination of human IgG4 antibodies against 88 food antigens 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, 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.

Principles of Testing

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.

Reagents And Materials Provided

1. 88 different nutritional antigens-coated microplate, 12 strips × 8-well, 4°C
2. Standards (0.35, 0.7, 3.5, 17.5, 50, 100 U/ml), 6 × 0.5 ml (ready to use) 4°C
3. Low positive Control, 0.5 ml (ready to use), 4°C
4. High positive Control, 0.5 ml (ready to use), 4°C
5. Sample dilution buffer, 40 ml (ready to use), 4°C
6. AP-antibody conjugate, 15 ml
7. 10× Wash Buffer, 60 ml, 4°C
8. PNPP substrate, 15 ml, 4°C (Protect from light)
9. STOP solution, 15 ml, 4°C

Materials Required But Not Supplied

Microplate reader capable of measuring absorbance at 450nm

Pipettes and pipette tips

Deionized or distilled water

Automated microplate washer (optional)

Storage

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

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

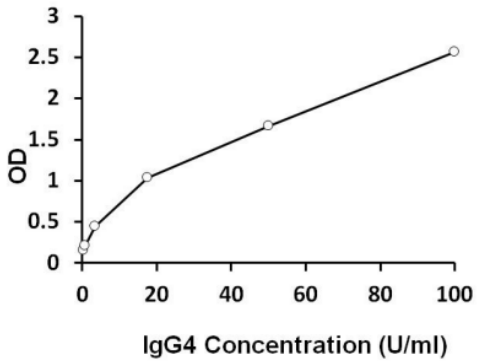
2. Add 100 µl of standards and samples in duplicate into wells.
3. Incubate for 60 minutes at 37°C.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× wash buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete 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

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 (×) 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.

Typical Standard Curve

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



Precision

Intra-assay precision: Egg white 7.7 %, Cow milk 8.0 %, Tomato 8.7 %

Inter-assay precision: Egg white 6.6-10.9 %, Cow milk 8.4-13.0 %, Tomato 4.6-7.4 %

Sensitivity

Analytical sensitivity: Egg white 0.22 U/mL, Cow milk 0.17 U/mL, Tomato 0.16 U/mL

Specificity

IgG

Recovery

Recovery: Egg white 90-107 %, Cow milk 89-103 %, Tomato 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

Precautions

1. Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
2. Store the kit at 4°C at all times.
3. Briefly spin down the AP-Antibody conjugate before use.
4. If crystals are observed in the 10× Wash buffer and Sample diluent buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
5. Ensure complete reconstitution and dilution of reagents prior to use.
6. It is highly recommended that the standards, samples and controls be assayed in duplicates.
7. Change pipette tips between the addition of different reagent or samples.
8. Samples contain azide cannot be assayed.