AccuDiag™ IgG Food Antigen Screen ELISA Kit

REF 5154-P1

IVD See External Label 2°C 8°C 96 Tests

IgG Food Antigen Screen ELISA	
Principle	**Indirect ELISA**
Detection	**Qualitative**
Sample	**50 µL serum/plasma**
Incubation Time	**150 minutes**
Shelf Life	**12 Months from the manufacturing date**

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

Diagnostic Automation, Inc. (DAI) IgG Screen 88 Food Antigens ELISA Test Kit has been designed for the detection and the quantitative determination of specific IgG antibodies against food antigens in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of DAI.

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.

SIGNIFICANCE AND SUMMARY

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.

ASSAY PRINCIPLE

The DAI IgG Screen 88 Food Antigens ELISA test kit is based on the principle of the enzyme immunoassay (EIA). 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 IgG 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 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 color 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 color.

SPECIMEN COLLECTION & 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 7 days. 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).

REAGENTS

Materials provided with the kit

The Diagnostic Automation, Inc. IgG Screen 88 Food Antigens ELISA Test Kit contains sufficient reagents for one patient (88 determinations). The first strip of the plate contains reference antigen (egg white, f01) for the generation of a calibration curve and the determination of controls.

1. Food/Reference antigen coated microtiter plate (1x)
2. Standards: 0.35, 0.70, 3.5, 17.5, 50, 100 U/ml (0.5 ml each)
3. Controls: weak positive, strong positive (0.5 ml each)
4. Anti-human IgG Enzyme Conjugate (15 ml)
5. Substrate (15 ml)
6. Stop Solution (15 ml)
7. Sample Diluent (40 ml)
8. Washing Buffer (10x) (60 ml)

Universal Reagents

Sample diluent, washing buffer and stop solution are identical for all IgG (4) food antigen screen test kits from DAI with Alkaline Phosphatase as detecting enzyme and may be interchanged between products and lots. All other reagents are assigned to a special kit lot and must not be mixed.

1. MT PLATE **Microtiter Plate**
   1 microtiter plate, coated with 88 food antigens (see distribution scheme) and 8x reference antigen (1. strip, color coded violet). Ready-to-use.
2. CAL **Standards A-F**
   6 x 0.5 mL, human plasma diluted with PBS/BSA, with 0.35, 0.70, 3.5, 17.5, 50 and 100 U/mL of IgG antibodies against egg white (f1). Addition of 0.05% sodium azide. Ready-to-use.
3. CONTR + **Weak Positive Control**
   0.5 mL, human plasma diluted with PBS/BSA, including low concentrations of IgG antibodies. Addition of 0.05% sodium azide. Ready-to-use.
4. CONTR + + **Strong Positive Control**
   0.5 mL, human plasma diluted with PBS/BSA, including high concentrations of IgG antibodies. Addition of 0.05% sodium azide. Ready-to-use.
5. CONJ **Anti-human-IgG Enzyme Conjugate**
   15 mL, mouse-a-human-IgG-AP, in proteinacious buffer solution. Addition of 0.01% methyl-isothiazolone, 0.01% bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.
6. SUBS **Substrate**
   15 mL, PNPP (Paranitrophenylphosphate). Ready-to-use.
7. STOP **Stop Solution**
   15 mL, 1 M sodium hydroxide. Ready-to-use.
8. SAMP DIL **Sample Diluent**
   40 mL, PBS/BSA buffer. Addition of 0.05% sodium azide. Ready-to-use.
9. WASH BUF CONC **Washing Buffer**
   60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with deionized water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

Materials required but not provided

1. 100 µL and 1000 µL micro- and multichannel pipettes
2. Microtiter Plate Reader (405 nm)
3. Microtiter Plate Washer
4. Reagent tubes for the serum dilution
5. Deionized water

REAGENT PREPARATION

Washing Solution: dilute before use 1+9 with deionized 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.
- A standard curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

ASSAY PROCEDURE

1. For each patient sample prepare one microtiter plate.
2. Pipet 100 µL each of the **diluted** (1:101) samples and the **ready-to-use** standards and controls respectively into the wells (see distribution scheme).
3. Cover plate and incubate for 60 minutes at 37°C.
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 and incubate for 30 minutes at 37°C.
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 and incubate for 60 minutes at 37°C.
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.

RESULTS

EVALUATION

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

Example

Standard	Class	OD-Value
0.35 U/mL	1	0.282
0.7 U/mL	2	0.390
3.5 U/mL	3	0.562
17.5 U/mL	4	0.923
50 U/mL	5	1.374
100 U/mL	6	2.108

The above table contains only an example, which was achieved under arbitrary 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 and controls of the IgG Screen 88 Food Antigens ELISA test kit are defined and expressed in arbitrary units (U/mL). This results

in an exact and reproducible quantitative evaluation. The 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 patient 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

IgG ELISA	Egg White	Cow's 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 %
Cross-Reactivity	No cross reactivity towards IgE up to 100000 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	85 %	100 %	78 %
Clinical Sensitivity	92 %	93 %	100 %

STORAGE CONDITIONS

Store kit components at 2-8°C. After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within three months.

LIMITATIONS OF THE ASSAY

- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking 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.
- 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 checkeregarding 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 there exists a danger of intoxication.

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

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Date Adopted	2023-07
Brand Name	AccuDiag™
REF 5154-P1	AccuDiag™ - IgG Food Antigens Screen ELISA
EC REP	CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands www.cepartner4u.eu
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