Version 28-04/25

User's Manual

IgG Food Screen 88 ELISA Kit

REF **DEIANS089**

Σ **15×1×96T**

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 simultaneous, quantitative measurement of food specific IgG antibodies against 88 allergens in human serum and plasma (EDTA, Citrate, Heparin).

General Description

It is intended to use as a tool to support the diagnosis of patients presenting various clinical symptoms associated with food intolerances. Advantage of the test is a non-invasive, quantitative measurement of plasma or serum samples for several foodstuff or –mixes at once. It is to be carried out by laboratory qualified personnel experienced in the use of in vitro diagnostic methods only. Lucretius once said that one man’s food may be another man’s poison. Individuals with allergies and other types of food sensitivities react adversely to certain foods and food ingredients that others can consume with no problems. Many different types of reactions are involved in these individual adverse reactions. Adverse food reactions may include IgE and non-IgE-mediated primary immunological sensitivities, non immunological intolerances (such as an enzyme deficiency or reactions to certain chemicals), as well as secondary sensitivities. Adverse immune reactions to foods which are not IgE mediated are often called food “intolerances.” The absence of IgE does not make them any less real; instead, other immune mechanisms, such as IgG or IgG4 antibodies are involved. IgG or IgG4-mediated adverse reactions to food are characterized by less severe reactions, are much more common (affecting approx. 45 % of the population) and have delayed onset (2 to 72 hours) after ingestion of an offending substance. Avoiding ingestion of such food (exclusion or rotation diet) is the best treatment to decrease symptoms. There are several evidences for the involvement of IgG or IgG4 in food 'intolerances' e.g.: • Allergic reactions may occur independently of antigen-specific IgE. • Subsequent decrease of IgG when the offending food is removed from the diet. • Specific serum IgG has been reported in cases of celiac disease, dermatitis, or atopic eczema, as well as in diseases with increased intestinal permeability, and inflammatory bowel disease (IBD). This evidence leads to the recommendation that IgG or IgG4-specific testing should be considered in cases where the patient shows unclear and chronic disorders, and in cases where classical diagnostics show no evidence.

Principles of Testing

The IgG Food Screen 88 ELISA is a conventional ELISA system (Enzyme Linked Immunosorbent Assay). Onto the surface of each cavity of a microtiter plate antigens from foodstuff or foodstuff mixes are immobilized. For the test, a serum or plasma sample is diluted and transferred to the microtiter well. Specific IgG (sIgG) antibodies will bind to the corresponding antigens of the foodstuff extract. Bound human IgG is detected by a specific horseradish peroxidase (HRP) conjugated anti human IgG antibody. After addition of the substrate TMB (3,3‘,5,5‘-Tetramethylbenzidine) bound IgG antibodies are visualized by a blue coloration of the solution. Addition of stop solution interrupts the reaction and the blue color turns into a yellow staining. Its intensity correlates with the amount of bound antibody. The yellow coloration is quantified via photometric measurement.

Reagents And Materials Provided

1. MTP Microtiter Plate, 15×1×96 Ready to use. Coated with 88 different nutritional allergens and 6 reference allergens. See chapter 19 for Plate layout. **2. ENZCONJ IgG** Enzyme Conjugate IgG, 1×225 mL Ready to use, yellow lid, goat-anti-human-IgG-HRP in proteinaceous buffer solution **3. CAL A-F** Calibrator A-F, 2×6×2 mL Ready to use, 0.35, 0.70, 3.5, 17.5, 50 and 100 U/mL. Contains: IgG antibodies against reference allergen, human plasma diluted with Diluent Buffer, <0.1% sodium azide. **4. NC/PC** Positive Control (PC) and Negative Control (NC), 2×2 mL See certificate of analysis for acceptable ranges. Ready to use. Contains: IgG antibodies against reference allergen, human plasma diluted with Diluent Buffer, <0.1% sodium azide. **5. DILUBUF** Diluent Buffer, 1×225 mL Ready to use, green lid. Contains: Phosphate buffer <0.1% sodium azide. **6. CONC WASH** Wash Buffer, Concentrate (20×), 3×250 mL White lid. Contains: PBS + Tween 20. **7. TMB SUBS** TMB Substrate Solution, 1×225 mL Ready to use, black bottle with black lid Contains: 3,3`,5,5`-Tetramethylbenzidine (TMB). **8. TMB STOP** TMB Stop Solution, 1×225 mL Ready to use, red lid. Contains 5% sulphuric acid.

Materials Required But Not Supplied

1. Disposable gloves 2. Timer 3. Graduated cylinder 4. sample tubes for dilution of the sample 5. Microliter pipette, Volume: 10-1000 µL and tips 6. Distilled water 7. Incubator 37°C 8. Microtiter plate photometer (450 nm) (reference wavelength 600-700 nm) 9. Recommended: Microtiter plate Washer

Storage

Store in a dark and cool place at 2-8°C / 36-46°F. All reagents (exception: wash buffer) are ready-to-use and packaged in bottles. The expiration date is printed on labels placed on the test packaging box. The expiration date of the kit is valid for all kit components, even if expiry of single components is different! After expiry all test components have to be discarded. In-use shelf life: at least 40 days at 2-8°C, but not longer than the expiry date.

Specimen Collection And Preparation

For performing the IgG Food Screen 88 ELISA human plasma (EDTA, Citrate, Heparin) or serum samples are required. Serum and plasma should be treated as potentially infectious. A pretreatment of the patient before blood withdrawal is not necessary. Please use standard laboratory procedures for processing of plasma or serum. Please pipet the samples and reagents with clean or sterile microliter pipets and disposable tips. Plasma or serum samples can be stored at 2-8°C for up to 14 days. For prolonged storage please keep at -20°C. Lipaemic, hemolytic or bacterial contaminated samples might lead to incorrect results. Furthermore, medication with immunomodulating drugs, innate or acquired immune deficiency or long or improper storage of the samples might influence the test results. **Note: The IgG Food Screen 88 ELISA can be used for the simultaneous, quantitative measurement of food specific IgG antibodies in capillary blood. For capillary blood collection Heparinized tubes should be used (e.g. Sarstedt Microvette 300 LH). Avoid excessive squeezing of finger tips for blood collection. Centrifuge immediately after collection.**

Plate Preparation

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL A 0.35 U/mL	Pineappel *Ananas comosus* f52	Cauliflower, raw *Brassica oleracea var. Botrytis* f62	Strawberry *Fradaria vesca* f44	Hazelnut *Corylus avellana* f17	Potato *Solanum tuberosum* f35	Prawn (Great king shrimp) *Panaeus monodon* f24	Maize, corn *Zea mays* f121	Peppermint *Mentha piperita* f126	Brussels sprouts *Brassica oleracea var. Gemmifera* f311	Spinach *Spinacia oleracea* f38	Walnut *Juglans rgia* f16
B	CAL B 0.7 U/mL	Anise *Pimpinella anisum* s1	Green sprouting broccoli *Brassica oleracea var. Italica* f182	Peanut *Arachis hypogaea* f13	Herring *Clupea harengus* f21	Casein *Bos primigenius f. taurus* f78	Milk, unboiled *Bos primigenius f. taurus* f199	Mackerel *Scomber scombrus* f171	Peach *Prunus persica* f53	Sheeps milk *Ovis aries* f325	Yellowfin tuna *Thunnus albacares* f40	White bean *Phaseolus vulgaris* f15
C	CAL C 3.5 U/mL	Apple *Malus domestica* f49	Buckwheat *Fagopyrum esculentum* f11	Rainbow trout *Oncorhynchos mykiss* f930	Raspberry *Rubus idaeus* f209	Cherry *Prunus avium* f73	Caraway *Carum carvi* s4	Almond *Prunus amygdalus* f20	Plum *Prunus domestica* f122	Pepper, black *Piper negrum* s7	Common thyme *Thymus vulgaris* s27	Wheat *Triticum arstivum* f5
D	CAL D 17.5 U/mL	Apricot *Prunus armeniaca* f168	Cheese, Camenbert *Bos primigenius f. taurus* f94	Gouda cheese *Bos primigenius f. taurus* f200	Millet *Panicus miliaceum* f164	Kiwi fruit *Actinidia deliciosa* f84	Salmon *Salmo salar* f41	Mango *Mangifera indica* f91	White mushroom (Button mushroom) *Agaricus bisporus* f141	Pork *Sus scrofa domestica* f26	Tomato *Lycopersicon esculentum* f25	Goat milk *Capra hircus* f300
E	CAL E 50 U/mL	Baker`s yeast *Saccharomyces cerevisiae* f45	Cashew nut *Anacardium occidentale* f204	Shaddock *Citrus maxima* f92	Chicken *Gallus gallus domesticus* f83	Garlic *Allum sativum* f47	Lamb meat *Ovis ammon f. aries, Ovis aries (Ovis spp.)* f88	Nutmeg apple *Myristica fragans* s5	Small radish *Raphanus sativus v. Sativus* f188	Celery *Apium graveolens* f85	Grape *Vitis vinifera* f50	Cinnamon *Cinnamomum verum* s8
F	CAL F 100 U/mL	Banana *Musa x paradisica* f29	Dill *Anethum graveolens* s14	Green bean (french) *Phaseolus vulgaris* f950	Cod fish *Gadus morhua* f3	Kohlrabi *Brassica oleracea var. gongylodes* f163	Leek *Allum porrum* f66	Orange *Citrus sinensis* f33	Rice *Oryza sativa* f9	Mustard seed *Sinapis alba* f89	Turkey meat *Meleagris gallopavo* f143	Lemon *Citrus limon* f32
G	Negative Control NC s11	Sweet basil *Ocimum basilicum* s11	Spelt *Triticum aestivum ssp. Spelta* f158	Cucumber *Cucumis sativus* f120	Camomille tea *Matricaris chamomillae* f96	Coconut *Cocus nucifera* f36	Lentil *Lens culinaris* f65	Bell pepper *Capsicum annuum* f46	Beef *Bos primigenius f. taurus* f27	Soy bean *Glycine max* f14	Vanilla *Vanilla planifolia* s9	Zucchini *Cucurbita pepo ssp. Pepo convar. Giromontina* f197
H	Positive Control PC f30	Pear *Parus communis* f30	Pea *Pisum sativum* f12	Oat *Averna sativum* f7	Carrot *Daucus carota* f31	Lettuce *Lactuca sativa* f194	Laurel (Bay leaf) *Laurus nobiles* s4	Parsley *Petroselinum crispum* f86	Rye *Secale cereale* f5	Asparagus *Asparagus officinalis* f132	Hen egg *Gallus gallus domesticus* f74	Onion *Allium cepe* f48

Reagent Preparation

The volumes stated below are for one run with one plate with one patient sample. **1. Preparation of Components**

Dilute / dissolve	Component	With	Diluent	Relation
50 mL	CONC WASH	950 mL	dist. water	1:20

2. Dilution of Samples

Sample	to be diluted	With	Relation	Remarks
Serum / Plasma either from venous or capillary blood	generally	DILUBUF	1:100	e.g. 110 µL + 10890 µL

Assay Procedure

Note: 1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only. 2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming. 3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents. 4. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells. 5. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells. 6. Storing the concentrated Wash Buffer at 2 - 8°C might lead to formation of salt crystals. If so, resolve those salt crystals by gentle warming (max 40°C) of the buffer solution prior the dilution. **Procedure:** 1. All reagents and the assay device must be adjusted to room temperature (18-30°C) for at least 30 minutes prior to use. 2. For each patient sample prepare one Microtiter Plate. The reference and control wells are located at the positions (A-H) of strip number 1, please consider the Plate Preparation. 3. Pipet 100 µL of the diluted samples and of the undiluted Calibrators with a microliter

pipet into the wells of the microtiter plate. 4. **Incubation: 60 minutes at 37°C** 5. Remove the samples, calibrators and controls from the microtiter plate via slapping the plate softly onto absorbent tissue. 6. Fill all wells with 300 µL diluted Wash Buffer by using a manual or automatic microtiter plate washer and remove the buffer immediately. Repeat this step three times. Afterwards remove residues of the washing buffer carefully from the bottom and rim of the cavities by slapping the plate softly onto absorbent tissue. 7. Pipet 100 µL Enzyme Conjugate IgG with a microliter pipet into each well. 8. Incubation: 30 minutes at 37°C 9. Fill all wells with 300 µL diluted Wash Buffer by using a manual or automatic microtiter plate washer and remove the buffer immediately. Repeat this step three times. Afterwards remove residues of the washing buffer carefully from the bottom and rim of the cavities by slapping the plate softly onto absorbent tissue. 10. Pipet 100 µL of the TMB Substrate Solution with a microliter pipet into each well. 11. Incubation: 15 minutes at 37°C 12. Pipet 100 µL TMB Stop Solution into the wells with the substrate solution. Do not wash the wells of the microtiter plate before this step. The Stop solution causes a color change from a blue solution to a yellowish solution. After stopping the reaction, the OD values change over time. Therefore, the measurement should be performed within 10 minutes after stopping the reaction. If calibrators and samples are measured on different plates, it must be taken into account that the elapsed time between STOP and measurement has to be the same for the to be compared plates. 13. Before measurement check for air bubbles in the liquid and scratches and dirt on the bottom of the microtiter plate. Measure at a wavelength of 450 nm with a microtiter plate photometer. A reference measurement at 600-700 nm is recommended.

Quality Control

The positive control contains specific IgG antibodies reactive to specified foods, whereas the negative control plasma does not contain measurable IgG antibodies. Controls are inserted like a normal sample and the test is carried out according to the instructions for use. It is good laboratory practice to run controls in appropriate time intervals. The supplied positive and negative controls as well as the standard series also serve as references for an internal functional control. The target values of standards and controls can be found in the certificate of analysis of the standard series. The values have to be calculated as described for the patient samples. The results are invalid if one or more measured values deviate from the target values after the test has been performed correctly. In this case the test has to be repeated. Reliable and reproducible results will be obtained when the assay is performed according to the procedural instructions and with adherence to good laboratory practices. The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

Calculation

For quantification of IgG and determination of reaction classes use the standard curve generated from the calibrator samples, followed by a calculated point-to-point method approach. The identified units/mL can be assigned to the respective classes and provide the level of specific IgG sensitization.

IgG class	U/mL	Intolerance	Position of Standards
0	0-0.35	None	
			CAL A
1	0.35-0.7	very low	
			CAL B
2	0.7-3.5	low-moderate	
			CAL C
3	3.5-17.5	moderate	
			CAL D
4	17.5-50	high	
			CAL E
5	50-100	very high	
			CAL F
6	≥100	extremely high	

In IgG diagnostics it is not usual to consider particular values as strictly defined limits. The level of IgG concentration is individually specific and cannot be classified into generalized limits. Table indicates the classification of the reactivity.* In order to diagnose a patient, the test results must always be evaluated together with the medical history and symptoms (anamnesis). **In contrast to the situation for type 1 allergies (immediate type) there is currently no uniform evaluation system for classifying food intolerances by means of IgG4 or IgG concentration.**

Reference Values

In general, higher test results indicate a higher concentration of the food specific IgG reacting to the food extracts or extract mixtures in IgG Food Screen 88 ELISA. Usually with all food specific IgG testing there is a potential for cross-reactivity between related and unrelated foods, which may contain similar or homologous molecules (antigens). The closer the biological relationship between different species, the greater is the degree of structural and immunological similarity of the epitopes present in the food. Accordingly, a patient who is clinically reactive to one species will likely be reactive to the other, closely related species due to immunological cross-reactivity of structurally related epitopes. On the other hand, cross-reactivity may occur also between biologically distantly related species. Some protein families are widely distributed and seem to contain highly conserved structures that can serve as similar epitopes.

Precision

Inter-assay precision Five tests were performed by the same person (Accuracy intraperson I Accuracy intertest) with 3 allergens. The coefficient of variation is 5-9% in dependency of the allergens. **Inter-person precision** Three tests were performed by 4 persons in quadruplicates, mean values of the tests of the same person were used for comparison to each of the other persons. Total number of tests: 48 wells. The mean coefficient of variation is 13.0%. **Inter-lot precision** Production of 3 different lots. Comparison of the test results of tests from these 3 ELISA KIT IgG lots. The decimal classes of the test for each allergen were compared to each of the other lots. 100 % of all tests show results within the tolerance of 1 class.

Sensitivity

Analytical sensitivity: For all foods- 0.35 U/mL Relative sensitivity: 81 %

Specificity

Relative specificity: 94 % (n=288) Compared to a CE-marked ELISA system for foodstuff specific human IgG. The IgG Food Screen 88 ELISA detects specific human IgG antibodies. Cross reactions with other immunoglobulin subtypes or species will not be expected. Reference ranges for blood serum concentrations of total IgG antibodies in the blood are described in the literature. These values do not apply to the values determined by the IgG Food Screen ELISA, since measures food-specific IgG antibodies. Determining reference ranges for food-specific IgG antibodies is not possible due to the high diversity of ingested foods by different individuals.

	Blood parameter	concentration	effect
Blocking effect at a concentration of 1.25 g/L IgG_4	IgA	3 g/L	no effect
	IgM	1 g/L	no effect
	IgG	30 g/L	no effect

Interferences

No known influences of HSA, Hemoglobin, Bilirubin, Cholesterol, Triglyceride, IgA and IgM at normal blood levels. No influences of Heparin, Na_3Citrat or EDTA in the sample have been experienced.

Precautions

1. For human in vitro diagnostic use only. For professional use only. 2. Please read the entire contents of these Instructions for Use before performing the test. 3. Do not use reagents beyond their expiration date. 4. In case of damage to the packaging please inspect the protective cover of the microtiter plate for damage. Make sure the reagents bottles are not damaged or open. In case of doubt do not use the test kit to avoid incorrect results or wrong diagnosis. 5. It is not recommended to pool any reagents or the same reagents from different lots. 6. The color reagent contains Tetramethylbenzidine; the stop solution contains sulfuric acid. Avoid contact with skin. Wear suitable protective gloves. 7. The IgG Food Screen 88 ELISA is for single use only. 8. Not following these precautions might lead to invalid test results. 9. Reagents, microtiter plates, calibrators and controls might be available separately or in different sizes. Store at 2-8°C until expiration date. Human material used in the IgG Food Screen 88 ELISA was tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.

Limitations

1. A definitive clinical diagnosis of food intolerance should not be based only on the results of a single diagnostic method. In vitro evidence of IgG should always be accompanied by full medical history and analysis of symptoms. 2. Each patient reacts individually, thus identical results in the test do not automatically imply the same diagnosis. Different foods with similar molecular structures or epitopes may trigger weak or severe cross-reactions. Cross-reactions must always be considered. 3. Negative in vitro results may occasionally occur in patients with food intolerance symptoms that clearly correlate with food contact. 4. Sensitization to foodstuffs not tested in the IgG Food Screen 88 ELISA cannot be excluded. 5. The binding capacity for IgG antibodies may vary from food to food. Therefore, identical results for different foods may not necessarily apply exactly equal IgG antibody levels.