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Instruction Manual

immuno^{LINE} IgG$_4$ Nutritional

Enzyme Immunoassay based on Nitrocellulose Test Strips
for the Detection of Human IgG$_4$ Antibodies against
20 Food Antigens in Serum and Plasma

CE

Cat. No.: ILE-LBL10
Storage: 2-8°C
For in-vitro diagnostic use only

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Symbole und Übersetzungen / Symbols and Translations

Symbol	English	French	German	Italian	Spanish	Greek
STRIP	Test strip	Membran	Teststreifen	Membrane	Tira de prueba	Δοκιμή της Γάζας
CONJ	Conjugate	Conjugué	Konjugat	Coniugato	Conjugado	Διάλυμα Συμπλόκου
SAMP DIL	Sample Diluent	Diluant échantillon	Proben-verdünner	Diluente del campione	Diluyente de muestra	Διάλυμα Αραίωσης Δειγμάτων
WASH BUF	Wash buffer	Tampon de lavage	Waschpuffer	Soluzione di lavaggio	Tampón de lavado	Πλυστικό Διάλυμα
CONC	Concentrate (<n>-fold)	Concentré (<n> fois)	Konzentrat (<n>-fach)	Concentrato (<n>-volte)	Concentrado (<n>-veces)	Συμπύκνωση (<n> φορές)
SUBS	Substrate	Substrat	Substrat	Substrato	Sustrato	Διάλυμα Υποστρώματος

1. Intended Use

The IMMUNOLAB immuno^{LINE} IgG_4 Nutritional test kit has been designed for the detection of specific food antigen-related IgG_4 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.

2. 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_4 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_4 for the diagnosis of food intolerances depends on the observation that some subclasses of IgG (mainly IgG_4) 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_4 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_4 intolerances show a delayed reaction of 24 to 120 hours and persistent symptoms may arise.

3. Principle of the Tests

The IMMUNOLAB immuno^{LINE} IgG_4 Nutritional test kit is based on the principle of the enzyme immunoassay (EIA). 16 patients can be tested with each kit. One test strip is required per patient. On each individual strip 20 different food antigens as well as a control line for test evaluation are coated in parallel lines. Following a pre-wetting step the strips are incubated with diluted patient serum. A binding between the IgG_4 antibodies of the serum and the immobilized food antigens takes place. After 30 minutes incubation at room temperature, the strips are rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG_4-AP conjugate is added and incubated for another 30 minutes at room temperature. After a further washing step, the substrate (BCIP/NBT) solution is pipetted and incubated for 60 minutes at room temperature, inducing the development of a precipitating dye on the lines in the case of positive reactions. The color development is terminated by rinsing the strips with wash solution. The concentration of the IgG_4 antibodies is directly proportional to the intensity of the color.

4. Limitations, Precautions and General Comments

- 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.
- None of the reagents are based upon human material. Nevertheless samples have to be treated as potentially infectious and 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 channels of the incubation tray 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 checked regarding the accuracy and precision. This refers amongst others to microliter pipets and other instrumentation (e.g. scanner for test evaluation).
- The contact of certain reagents, above all the substrate with skin, eye and mucosa has to be avoided.

5. Reagents Provided

Components	Volume / Qty.
STRIP Food antigen coated nitrocellulose test strips	16
CONJ Enzyme Conjugate	18 mL
SAMP DIL Sample Diluent	60 mL
WASH BUF CONC Washing Buffer (10×)	60 mL
SUBS Substrate	18 mL
Incubation Tray	1
Evaluation Template	1

Storage and Stability (refer to the expiry date on the outer box label)
Store kit components at 2-8°C. After use, the bottle caps should be replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within six months.

Universal Reagents
Washing buffer, sample diluent and substrate are identical for all immuno^{LINE} IgG or IgG_4 test kits from IMMUNOLAB 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.

5.1. STRIP **Nitrocellulose Test Strips**
16 strips for 16 patients. Each individual strip is coated with 20 different food antigens (see Distribution Scheme) and one control antigen. Ready-to-use.

5.2. CONJ **Enzyme Conjugate**
18 mL, anti-human-IgG_4-AP (mouse), in protein-containing buffer solution. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.

5.3. SAMP DIL **Sample Diluent**
60 mL, PBS/BSA buffer. Addition of 0.05 % sodium azide. Ready-to-use.

5.4. WASH BUF CONC **Washing Buffer**
60 mL, TBS + 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. Diluted washing buffer can be stored at 2-8°C for 4 weeks.

5.5. SUBS **Substrate**
18 mL, BCIP/NBT. Ready-to-use.

5.6. Incubation Tray
With 8 channels for the incubation of 8 test strips.

5.7. Evaluation Template

5.8. Instruction Manual

6. Distribution Scheme

One control line as well as 20 food antigens are distributed over the test strip in the following order.

CL	Control
1	Egg
2	Cow's milk
3	Wheat
4	Corn
5	Rice
6	Soy
7	Peanut
8	Sesame
9	Shrimp
10	Crab
11	Cod
12	Pork
13	Tomato
14	Potato
15	Carrot
16	Orange
17	Kiwi
18	Strawberry
19	Banana
20	Apple

7. Materials Required but not Provided

- 20 µL- and 1000 µL micropipets
- Tubes for serum dilution
- Rocking shaker
- Vacuum pump
- Deionized water

8. 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 (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 have to be diluted 1:51 with ready-to-use sample diluent (e.g. 20 µL serum + 1 mL sample diluent). Thus, for the 20 tests per patient screen only 20 µL serum are necessary.

9. Assay Procedure

9.1. Preparation of Reagents

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.

9.2. Assay Steps

1. Place the required amount of test strips into the incubation tray. The visible marking on each test strip has to be directed upwards.
2. Fill 1 mL sample diluent into each channel and incubate the test strips on a rocking shaker for 5 minutes at room temperature. Aspirate the solution with the aid of a vacuum pump afterwards.
3. Fill 1 mL 1:51 diluted sample into each channel of the tray and incubate the test strips on a rocking shaker for 30 minutes at room temperature. Aspirate the solution with the aid of a vacuum pump afterwards.

   Note: For an easier handling the sample can be diluted and mixed directly in the incubation channel. For this fill 1 mL sample diluent into the channel and add 20 µL sample. Start shaking immediately after sample addition.
4. Fill 2 mL diluted washing solution into each channel and rinse the strips for at least 2 minutes on a rocking shaker at room temperature. Aspirate the solution with the aid of a vacuum pump afterwards. Carry out this step three times altogether.
5. Fill 1 mL ready-to-use conjugate into each channel and incubate the test strips on a rocking shaker for 30 minutes at room temperature. Aspirate the solution with the aid of a vacuum pump afterwards.
6. Repeat the washing step as described in point 4.

7. Fill 1 mL ready-to-use substrate into each channel and incubate the test strips on a rocking shaker for 60 minutes. Aspirate the solution with the aid of a vacuum pump afterwards.
8. Fill 1 mL diluted washing solution into each channel to terminate the substrate reaction. Aspirate the solution with the aid of a vacuum pump.
9. Air dry and evaluate the test strips.

Note: The speed of the rocking shaker used in all incubation steps is dependent on the geometry of the individual instrument. Validation experiments showed that a speed of >90/min resulted in reliable results with common devices.

10. Evaluation

Moist test strips may show a certain background staining which disappears while drying. For this reason the strips are only evaluated when they are completely dry.

Note that some samples show a background staining, which will not disappear while drying. In this case white bands may occur at the position of the coated antigens. These signals have to be interpreted as negative.

Some samples show very weak reactions to multiple antigens. It cannot be totally excluded that these samples tend to unspecific reactions and therefore they should to be considered as negative for all antigens with similarly weak reactions.

The test run has to be considered as valid when a clear visible control line develops during substrate incubation. This line will appear even if all food antigens show a negative result. The control line is the nearest signal to the identification code which is directly printed on the test strip.

10.1. Visual Method

The control line of the dried test strips is aligned to the marking on the template (“CL”). Lines 1-20 indicate the location of the coated antigens according to the order as stated in the “Distribution Scheme” section. Signals are interpreted for each antigen according to the following scheme:

Class	Signal	Interpretation
0	no signal or weak signals for multiple antigen	negative
1	weak signal	borderline result
2	moderate signal	positive result
3	intense signal	strong positive result

10.2. Scanner Method

The IMMUNOLAB immuno^{LINE} IgG_4 Nutritional test can be evaluated using a scanner in combination with IMMUNOLAB’s *immuno^{LINE} Evaluation Tool* software to get quantitative results. Please refer to the instruction manual of the software.

11. Assay Characteristics

Spec. IgG_4 Lineblot	Egg white	Cow's milk	Wheat	Peanut
Intra-assay precision	*8.9 – 16.5%*	*4.4 – 8.2%*	*4.3 – 24.4%*	*8.9 – 14.6%*
Inter-assay precision	*3.6 – 25.7%*	*3.5 – 16.5%*	*5.1 – 29.1%*	*2.0 – 10.3%*
Inter-lot precision	*5.4 – 34.4%*	*4.5 – 18.6%*	*5.1 – 29.1%*	*2.0 – 10.3%*
Recovery	*105 – 113%*	*120 – 129%*	*90 – 107%*	*90 – 107%*
Linearity	*93 – 119%*	*93 – 111%*	*116 – 127%*	*97 – 114%*
Cross reactivity	No cross reactivity towards other Ig isotopes			
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	93%	88%	95%	91%
Clinical sensitivity	96%	94%	94%	94%

12. Short Instruction

1. Reaction: Diluent	**1 mL**	Sample Diluent
	5 min ↓ at room temperature	
2. Reaction: Sample	**1 mL**	Patient sample (diluted 1:51)
	30 min ↓ at room temperature	
	Wash 3x, 2 mL washing buffer (diluted 1:10)	
	3x2 min ↓ at room temperature	
3. Reaction: Conjugate	**1 mL**	Conjugate (ready-to-use)
	30 min ↓ at room temperature	
	Wash 3x, 2 mL washing buffer (diluted 1:10)	
	3x2 min ↓ at room temperature	
4. Reaction: Substrate	**1 mL**	Substrate (BCIP/NBT, ready-to-use)
	60 min ↓ at room temperature	
	1 mL	Washing buffer (diluted 1:10)
	↓	
	Evaluate	

13. References

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