INSTRUCTION MANUAL

REF **5120**

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sIgG$_4$ Allergy LINE 1

- 24 determinations -

CE

IVD *In vitro* diagnostic device

Line Immunoassay for the determination of specific IgG$_4$ antibodies to foods / food mixtures in human serum or plasma

REF	Catalogue number	**LOT**	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction	D	Biological risk

GA GENERIC ASSAYS GmbH

Ludwig-Erhard-Ring 3

15827 Dahlewitz, Germany

Telephone: +49 (0) 33708-9286-0
Fax: +49 (0) 33708-9286-50

www.genericassays.com

INTENDED USE

sIgG$_4$ Allergy LINE 1 is used for the parallel determination of specific IgG$_4$ antibodies against foods and food mixtures in human serum or plasma for the diagnosis of food allergies and intolerances.

The test is used to support the diagnostic process in patients suspected to have food allergies. The assay permits a large number of tests to be conducted in parallel over a short period of time. It must be performed by qualified professionals familiar with in vitro diagnostic methods.

PRINCIPLE of the TEST

sIgG$_4$ Allergy LINE 1 is a Line Immunoassay based on ELISA test system (enzyme-linked immunosorbent assay) for the determination of specific IgG$_4$ antibodies to food allergens in human serum or plasma.

One test strip contains 27 food extracts/mixtures and 3 standards which are embedded in the membrane.

Specific IgG$_4$ antibodies (sIgG$_4$) from the specimen bind to the respective food antigens on the membrane. The bound human IgG$_4$ is detected by a specific anti-human IgG$_4$ antibody that is conjugated to the horseradish peroxide enzyme.

After adding a colour substrate, bound IgG$_4$ antibodies are identified by an indigo precipitate.

The individual degree of sensitisation can be determined quantitatively by using a separately available reader or semiquantitatively with the naked eye in the case of all food extracts/food mixtures, and assigned to sIgG$_4$ classes. The result is read out by comparing the colour intensity of the signal against the standard signals on the strip assay.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can also be used.

The samples may be kept at 2 - 8 °C for up to 14 days. Long-term storage requires - 20 °C.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Dilute each of the specimens at a ratio of **1:20**, i.e. 75 µl serum or plasma with 1.425 ml wash buffer (made from B) directly in the incubation tray. The dilution can also prepared before, the assay than is started using 1.5 ml of prediluted sample.

TEST COMPONENTS for 24 determinations

A Ag 24	**LINE strips** 24 numbered strips, coated with 30 reaction lines and green colour code: - 27 lines coated with food or food mixtures - 3 Standard lines	24 strips for the determination of 27 antibody specificities each
B WASH 20x	**Wash buffer** sufficient for 800 ml solution	40 ml concentrate capped white
C CON G4	**Conjugate** anti-human IgG_4, coupled with HRP	40 ml ready for use capped yellow
E SUB	**Substrate** 3,3',5,5'-tetramethylbenzidine	40 ml ready for use capped blue
F STOP	**Stop solution** Tris/Phosphate buffered solution, pH 3.2	40 ml ready for use capped black
G	**Incubation tray** for 8 test strips	3
H	**Plastic tweezers**	1
I	**Interpretation template** for glueing of processed strips	1

Allergens coated on Allergy LINE $sIgG_4$ Panel 1:

f4	Wheat	f84	Kiwi fruit
f5	Rye	f29	Banana
f7	Oat	f49	Apple
f11	Buckwheat	f25	Tomato
f31	Carrot	f35	Potato
f48	Onion	f89	Mustard
f13	Peanut	f3	Cod fish
f14	Soy bean	f41	Salmon
f950	Green bean	f24	Shrimp
f74	Egg	f141	Button mushroom
f199	Milk	f45	Baker´s yeast
f325	Sheeps milk	f26	Pork meat
f9	Rice		
f17	Hazelnut		
f20	Almond		

Materials required in addition

- adjustable micropipettes
- pipette tips
- horizontal or rocking shaker
- graduated cylinders
- distilled or deionised water
- timer
- paper towel

Size and storage

$sIgG_4$ Allergy LINE 1 has been designed for 20 x 2 determinations.

The expiry date of each component is reported on its respective label, that of the complete kit on the box label.

Upon receipt all components of the $sIgG_4$ Allergy LINE 1 have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

Take strips with a plastic pincer only.

Prepare a sufficient amount of wash solution by diluting the concentrated washing buffer 20 times (1 + 19) with deionised or distilled water. For example, dilute 40 ml of the concentrate with 760 ml of deionised or distilled water.

For each test strip 10.5 ml of washing buffer are requested.

The wash solution prepared is stable at 2 - 8 °C up to 30 days.

All other components are ready for use and so stable until the expiry date.

Move the incubation tray on a shaker at 50 rpm. Ensure that the strips are immersed completely.

Avoid exposure of the substrate solution to light.

ASSAY PROCEDURE

Predilute samples at a ratio of **1:20**, i.e. 75 µl sample with 1.425 ml wash buffer (made from B), or preparation of sample dilution directly within the incubation tray.

1. Bring all reagents to room temperature (RT) (18-25°C) before use. Mix gently without causing foam.
2. Place the strips (A) with the reactive side up (labels on top) into the respective well. Dispense 1.5 ml of diluted samples into the respective wells (or prepare sample dilution directly in the tray). Completely immerse the test strips.
3. Incubate **30 min** at RT (18-25°C) while shaking (about 50 rpm)..
4. Decant or aspirate, wash each well **3 times** for **3 min** with **1.5 ml** wash solution (made of B) while shaking. (Discard the solution in the wells by slowly inverting the plate. Dry the edges of the tray with absorbent paper in order to remove the remaining fluid.)
5. Add 1.5 ml conjugate (C) to each well
6. Incubate **30 min** at RT (18-25°C) while shaking (about 50 rpm).
7. Decant or aspirate (see 4.) and wash each well **3 times** for **3 min** with **1.5 ml** wash solution (made of B) while shaking.
8. Add 1.5 ml of substrate (E) to each well.
9. Incubate **5 min** at RT (18-25°C) while shaking (about 50 rpm).
10. Add 1.5 ml of stop solution (F) to each well.
11. Incubate **5 min** at RT (18-25°C) while shaking (about 50 rpm).
12. Dry the strips: Collect the strips from the wells and put them onto absorbent paper with the **reactive side upwards**. After approximately **15 - 30 min** the strips are to be interpreted using the interpretation template.

EVALUATION OF RESULTS

Evaluation:

Results should be interpreted only after strips have been dried for about 15 to 30 minutes.

Each test strip contains methodological controls (standards). They appear as coloured bands after the test has been performed. If standards are missing, the result for the given test strip will be invalid and the test will need to be repeated.

If using a separately available reader, the results can be analyzed quantitatively. To do so, place the test strips in the reader and read out the results using the accompanying software. By comparing the intensities of the test and standard bands, the results can be determined in kUA/L and by class. Note the instructions in the corresponding manual. To evaluate the results semi-quantitatively, compare the intensities of the standards against those of the individual foods/food mixtures. See Table 1.

Correlation between Allergy LINE class and food-specific IgG_4:

Class	kUA/L	Result
0	< 0,35	negative, no clinical significance
Standard 1		
1 – 2 (+)	≥ 0,35 < 3,5	low food-specific IgG_4 concentration
Standard 2		
3 – 4 (++)	≥ 3,5 < 50	moderate food-specific IgG_4 concentration
Standard 3		
5 – 6 (+++)	≥ 50	high food-specific IgG_4 concentration

Interpretation of results:

In diagnostic testing for food intolerance, it is not customary to interpret measurements within stringently defined limits. The $sIgG_4$ concentration is very individual and cannot be fixed within precise limits*. To make a diagnosis, the test results must always be considered in conjunction with the symptoms and medical history.

Limitations of Method

1. A conclusive clinical diagnosis should not be based solely on the results of a single diagnostic procedure. In addition to the in vitro determination of specific IgG_4, a thorough medical history should be taken and the various symptoms investigated.
2. Every patient reacts differently. Hence, identical results delivered by the test will not automatically lead to the same diagnosis. Diverse food extracts with similar molecular structures or epitopes can trigger weak or strong cross-reactions which must always be taken into account.
3. Occasionally, negative in vitro results can also be obtained in patients with symptoms which are clearly correlated to contact with certain food extracts.
4. Reactions to foods other than those contained in the Allergy LINE test cannot be ruled out.
5. Patients with clinical symptoms and Allergy LINE class 0 reactions to the respective foods should be referred to a specialist for further investigation.
6. The binding capacities of specific IgG_4 antibodies can vary from food to food. Therefore, identical classes for various foods will not necessarily correspond to the same $sIgG_4$ content.
7. Reliable and reproducible results are achieved if the test is performed in accordance with the methodological instructions and good laboratory practice.

Expected results:

As a rule, the higher the class that is determined, the higher the concentration of food-specific IgG_4 against the respective food in Allergy LINE. As with all $sIgG_4$ tests, cross-reactions can occur between related and unrelated foods containing similar or homologous molecules (antigens). The closer the biological relationship between the different types, the greater the degree of structural and immunologic similarity between the foods of both species.

In light of the immunologic cross-reactions of structurally related foods, therefore, a patient exhibiting a clinical reaction to one food is likely to also react to foods that are closely related. On the other hand, cross-reactions can also occur between species that biologically are distantly related. Some protein families are widespread and contain highly preserved structures that can function as largely consistent epitopes.

CHARACTERISTIC ASSAY DATA

Analytical Sensitivity

$sIgG_4$ Allergy LINE 1 detects specific IgG_4 antibodies. No cross-reactivity with other immunoglobulin species is known.

Diagnostic Specifity and Sensitivity

The test results obtained with Allergy LINE have been evaluated in comparison with $sIgG_4$ ELISA lab systems. There are no known limitations or interferences.

Precision

Inter-assay precision: Duplicate assays were performed on control serum to determine inter-assay precision. The tests were performed by five different individuals on two consecutive days. The mean values (MV) and coefficients of variation (CV) were determined separately for each allergen. Values below 10% are accepted for the inter-assay precision of all allergens with positive signals. This specification criterion is not appropriate for describing the precision of allergens with negative signals. The result is accepted if the analysis is consistent across all 20 measurements.

Allergen code	Number of Measurements	MV (RAST)	CV (%)	Analysis
f5	20	5,8	2,2	Positive
f13	20	5,5	1,0	Positive
f950	20	4,0	5,4	Positive
f29	20	3,1	6,6	Positive
f325	20	5,1	1,0	Positive
f199	20	4,1	5,4	Positive
f35	20	0,4	7,1	Negative
f24	20	5,4	1,7	Positive
f11	20	2,6	6,0	Positive
f20	20	4,0	6,0	Positive

INCUBATION SCHEME

sIgG$_4$ Allergy LINE 1 (5120)

Dilution of patient samples 1:20, e.g. 75 µl sample with 1.425 ml wash buffer (made from B), or sample dilution directly in the incubation tray (step 2)

1.	Bring all reagents and the requested number of strips to room temperature (18-25°C)	
2.	Place the strips with the reactive side upside in the respective tray and dispense 1.5 ml of diluted sample	
3.	Incubate while shaking	30 minutes RT (18-25°C)
4.	Decant, wash strips while shaking	3 x 3 min with 1.5 ml (made of B)
5.	Pipette 1.5 ml conjugate (C) in the respective well	
6.	Incubate while shaking	30 minutes RT (18-25°C)
7.	Decant, wash strips while shaking	3 x 3 min with 1.5 ml (made of B)
8.	Pipette 1.5 ml substrate (E)	
9.	Incubate while shaking	5 minutes RT (18-25°C)
10.	Pipette 1.5 ml stop solution (F)	
11.	Incubate while shaking	5 minutes RT (18-25°C)
12.	Put the strips **reaction side up** onto absorbent paper to dry. After approximately 15 - 30 min the strips are ready to be interpreted	

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for re-constituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C prior use in the original shipping container.
- Some of the reagents contain small amounts of Neolone M10 (< 1.0 % v/vw) as a preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Since the kit contains potentially hazardous materials the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

REFERENCES

1. Hardman G, Hart G: Dietary advice based on food-specific IgG results. Nutrition & Food Science 2007; 37(1): 16-23.
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3. David TJ: Adverse reactions and intolerance to foods. Br Med Bull 2000; 56: 34-50