EXPORT ONLY VERSION

Allerquant™ 90 Foods IgG ELISA Kit

REF 7180

For Qualitative Analysis of Antibodies to 90 Food Allergens in Human Serum

October 2020

BIOMERICA

I. INTENDED USE

The *Allerquant™ 90 Foods IgG Elisa Kit* is for measuring the relative amount of food-specific IgG antibody in human serum. The values obtained must always be correlated with the clinical presentation, since elevation of a certain food-specific antibody by itself does not necessarily mean disease. The kit can be used to help diagnose hidden food intolerance as well as to develop elimination diets which along with other clinical information can help in the treatment of Irritable Bowel Syndrome (IBS) and other diseases caused by food intolerance/allergies. This kit does not provide information about IgE-mediated allergies.

II. BACKGROUND

The topic of food intolerance and the role of food and food additives as causative factors in food hypersensitivity diseases have prompted considerable interest for many years. Numerous studies have suggested that delayed food allergies (IgG-mediated) may lead to chronic illnesses. Continuous consumption of an offending food may result in a weakened immune system, which will enable illnesses to develop. Among some of the more serious diseases that have been linked to food intolerance are Irritable Bowel Syndrome (IBS), Crohn's Disease, Ulcerative Colitis, Celiac Disease, Asthma, Rheumatoid Arthritis, Psoriasis, Rosacea, and Eczema. Commonly implicated foods include cow's milk, eggs, wheat, corn, chocolate, nuts, soybean, and shellfish. Most of the foods that belong to one group may share common allergenic properties, and sometimes foods of two different groups may also show cross-reactive allergic reactions. To reduce certain food intolerance reactions, cooked foods may be recommended because cooked food can be less allergenic than raw food.

Most common food allergy symptoms are gastrointestinal-related and may include nausea, diarrhea, constipation, bloating, and abdominal pain. The clinical manifestations of food intolerance may also include dermatological, neurological, muscular-skeletal, or respiratory symptoms more commonly associated with classic allergy (IgE) disorders. The role of food intolerance in conditions such as migraine headaches and allergic tension-fatigue syndrome is still somewhat controversial. It is important to remember that the symptoms of food intolerance, especially gastrointestinal symptoms, can be mimicked by a variety of other conditions. This test is often used to assist in diagnosis when other clinical manifestations preclude a definitive diagnosis.

IgG-mediated food intolerance is usually treated by dietary avoidance of the offending food(s). Studies have shown that avoidance generally results in decreased symptoms. Furthermore, one study showed that 38% of foods originally producing symptoms were well tolerated in the diet after one-two years of avoidance. Thus, in general, a diet that excludes offending foods may help to relieve many symptoms associated with intolerance and may in fact, lead to the ability to reintroduce the offending foods back into the diet after a one to two year period.

III. PRINCIPLE OF THE TEST

Specific allergens are immobilized separately onto microtiter wells. The allergens are allowed to react with specific antibodies present in the patient's serum. Excess serum proteins are removed by the wash step. Enzyme labeled antibody conjugate is allowed to react with allergen-antibody complex. A color is developed by the addition of a substrate that reacts with the coupled enzyme. The color intensity is measured and is directly proportional to the concentration of IgG antibody specific to a particular allergen.

IV. REAGENTS AND MATERIALS

This test kit contains sufficient wells and reagents to assay 3 patient sera for antibodies to 90 different foods.

PLA FOOD = 90 Foods Coated Microwells ..... 3 plates
DIL SPE 1X = Sample Diluent (Green) ..... 1 x 56 ml
BUF WASH 66.67X = Wash Buffer (concentrate) ..... 1 x 30 ml
CAL FOOD IgG = Foods IgG Calibrator ..... 1 x 1.0 ml
CTRL + IgG = Foods IgG Positive Control ..... 1 x 1.0 ml
CONJ ENZ IgG-HRP = Foods IgG-HRP Conjugate ..... 1 x 40 ml
SUBS A TMB = Substrate Solution A (TMB) ..... 2 x 12 ml
SUBS B H_2O_2 = Substrate Solution B (hydrogen peroxide) ..... 2 x 12 ml
SOLN STOPPING = Stopping Solution (1N H_2SO_4) ..... 1 x 20 ml

V. WARNINGS AND PRECAUTIONS

1. **Potential Biohazardous Material**

The source of the Calibrators and Controls is human serum. The human serum used has been found non-reactive to HbsAg, anti-HIV 1/2 and anti-HCV when tested with FDA licensed reagents. Because there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled as if potentially infectious

2. **Sodium Azide**

Some reagents contain sodium azide as a preservative. Sodium azide may react with lead, copper or brass to form explosive metal azides. When disposing of these materials, always flush with large volumes of water to prevent azide buildup.

3. **Stopping Solution**

Stopping Solution consists of 1N H_2SO_4. This is a strong acid and should be handled with caution. It can cause burns and should be handled with gloves. Wear eye protection and appropriate protective clothing. Avoid inhalation. Dilute a spill with water before absorbing the spill with paper towels.

4. **Substrate Solution A**

Substrate Solution A contains Dimethyl Sulfoxide (DMSO). DMSO is a skin irritant and can also cause irritation to the mucosal membranes and upper respiratory tract if inhaled, or ingested. Avoid exposure by wearing personal protective equipment such as gloves and safety glasses. If skin or eye contact occurs, flush with water for a minimum of 15 minutes. If inhaled, move to fresh air. If ingested, obtain medical attention.

A sulphurous odor may be present in the Substrate Solution A and will not affect ELISA results. The handling of Substrate Solution A and preparation of the Working Substrate Solution should be done in a fume hood or a well-ventilated area to minimize exposure.

VI. PREPARATION OF PATIENT SAMPLE

Dilute patient's serum 1:100 in Serum Diluent. Take 0.1 ml (100 µl) of patient serum and add it to 10 ml of Serum Diluent. Mix thoroughly.

VII. REAGENT PREPARATION AND STORAGE

1. **Wash Buffer:** If crystals are present in the Wash Buffer concentrate due to storage at a lower temperature such as 2-8°C, dissolve by placing the vial in a 37°C water bath or incubator for 30 minutes. Wash the contents of the vial into a 2000 ml flask with DI water and Q.S. to 2000 ml mark with DI water. Label it as Working Wash Buffer and store refrigerated at 2-8°C. The Working Wash Buffer is stable for 6 months at 2-8°C.

2. **Substrate Solution:** Mix Substrate Solution "A" and "B" in equal proportions 30 minutes before use. (*For example mix 6 ml each of "A" and "B" for each 20 patients or plate to be used).* Discard the unused substrate mix solution. Do not interchange the caps on these solutions. If the mixed substrate solution looks blue in color before use, it should be discarded. Mixed substrate solution is stable for 60 minutes at room temperature and should be kept away from light until use.

VIII. ASSAY PROCEDURE

Bring all the test kit reagents to room temperature before use.

1. ***PREPARATION OF CALIBRATION CURVE:*** Label four 12 x 75 mm glass tubes as 50, 100, 200 & 400 U/ml. Dispense 150 µl of Serum Diluent into these four tubes. Add 150 µl of Food Calibrator to the tube labeled 400 U/ml. Mix and transfer 150 µl into tube labeled 200 U/ml. Mix and transfer 150 µl into the tube labeled 100 U/ml. Again mix and transfer 150 µl into the tube labeled 50 U/ml. At this point you should have 150 µl in tubes 100, 200 & 400 U/ml, and 300 µl in tube 50 U/ml. This is the calibration curve to be used in the assay. Transfer 100 µl from each of these tubes to the microplate as follows.

Tube Label	Well Label
50 U/ml	1B
100 U/ml	1C
200 U/ml	1D
400 U/ml	1E

Add 100 µl of Serum Diluent to well 1A for the blank wells and 100 µl of Positive Control to well 1F.

2. Place 100 µl of the diluted patient serum (**see Preparation of Patient Sample – Section VI)** into all the other wells. There should be 100 µl of liquid in all the wells.
3. Cover the plates with parafilm or plastic wrap and incubate at room temperature (22-25°C) for 1 hour.
4. After one-hour incubation, wash all the microwells three times with 300 µl of working wash buffer each time **(see Reagent Preparation – Section VII).** If you use an automated washer, check the manufacturer's instructions for a three-cycle wash procedure with 300 µl wash volume. Blot thoroughly after wash step.
5. Add 100 µl of Food IgG-HRP Conjugate to all the wells.
6. Incubate the plates for 30 minutes at room temperature (22-25°C).
7. Wash the plates again as in step #4.
8. Add 100 µl of Working Substrate mix to all the wells **(see Reagent Preparation – Section VII).**
9. Cover the plates and incubate for 10 minutes at room temperature (22-25°C).
10 Add 50 µl of Stopping Solution to all the wells (blue color in the wells will change to yellow). Mix the reagents by gently tapping the plate.
11. Set the microplate reader at 450 nm and read the absorbance in all the wells.

IX. CALCULATION OF RESULTS

Automated Method

Use an automated regression program to reduce the data. Acceptable results will be obtained by using (1) Cubic Spline, (2) Point to Point, or (3) Quadratic. Interpolate the patient values from the calibration curve.

Manual Method

- Determine the absorbance for each calibrator, control, and sample.
- Construct a dose response curve where the absorbance for each calibrator is plotted against the concentration of the corresponding calibrator on a semi-log or linear graph paper. Draw a straight line between 2 adjacent points. This mathematical algorithm is commonly known as the "point-to-point" calculation.
- Read off sample concentrations directly from the curve.

X. QUALITY CONTROL

For the test to pass, it must meet the following Q.C. specifications for O.D. (Optical Density) at 450 nm.

O.D. BLANK	< 0.2
O.D. 50 CAL	> 1.2 x OD BLANK

O.D. 100 CAL	> 1.2 x OD 50 CAL
O.D. 200 CAL	> 1.2 x OD 100 CAL
O.D. 400 CAL	> 1.2 x OD 200 CAL
Concentration Positive	> 100 U/ml

XI. INTERPRETATION OF RESULTS

The absorbance readings, after extrapolation as Biomerica U/ml, should be interpreted as follows for each allergen or extract.

READING	INTERPRETATION	
< 50 U/ml	Negative	**0**
50 - 100 U/ml	Mildly Intolerant	**+1**
100 - 200 U/ml	Moderately Intolerant	**+2**
> 200 U/ml	Highly Intolerant	**+3**

XII. REFERENCES

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XIII. SYMBOLS

	Storage Temperature
LOT	Lot Code
	Expiration
	Manufacturer
	Caution, see instructions
IVD	For in vitro diagnostic use
REF	Catalog No.

XIV. ORDERING INFORMATION

Additional IgG mediated food intolerance screening products are available from Biomerica in several different configurations. For more information about these test configurations, contact Customer Service.

Contact:

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2℃ 8℃

IVD

CE

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EC REP

according to IVDD 98/79/EC
MDSS GmbH
Schiffgraben 41
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90 Foods (1 Patient) Microplate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	*BLANK*	Apple	Butter	Cheddar Cheese	Cola nut	Garlic	Lettuce, Iceberg	Oat	Pinto bean	Sardine	Strawberry	Trout
B	Calibrator 1	Mango	Cabbage	Chicken	Corn	Goat's Milk	Lemon	Olive	Pineapple	Scallop	String bean	Tuna
C	Calibrator 2	Banana	Cane sugar	Chili Pepper	Cottage Cheese	Grape, white/concord	Hair Tail	Onion	Pork	Sesame	Sunflower seed	Baby Bok Choy
D	Calibrator 3	Barley, whole grain	Cantaloupe	Chocolate	Cow's Milk	Grapefruit	Lobster	Orange	Potato	Shrimp	Sweet potato	Walnut, black
E	Calibrator 4	Beef	Carrot	Cinnamon	Crab	Green pea	Malt	Oyster	Rice	Allium fistulosum	Caraway	Wheat
F	Positive Control	Blueberry	Cashew	Clam	Cucumber	Green pepper	Millet	Parsley	Rye	Soybean	Tea, black	Turkey
G	Almond	Broccoli	Cauliflower	Codfish	Egg, white/yolk	Mutton	Mushroom	Peach	Durian	Spinach	Grass Carp	Yeast, (Sac. cer.)
H	Watermelon	Buckwheat	Celery	Coffee	Eggplant	Honey	Mustard Seed	Peanut	Salmon	Squashes	Tomato	Yogurt

7180 October 2020

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