

# Allerquant™ 90 Foods IgG ELISA Kit

REF E-170

For Semi-Quantitative Analysis of Antibodies to 90  
Food Allergens in Human Serum



## I. INTENDED USE

The *Allerquant 90G IgG Elisa Test* 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 a considerable interest for many years. Commonly implicated foods include cow's milk, eggs, wheat, corn, chocolate, nuts, soybean, and shellfish. About 0.5% of infants may have a hypersensitivity reaction to cow's milk. Research studies suggest that in a child with proven food hypersensitivity, the probability of food hypersensitivity in subsequent siblings is increased up to 50%. 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, and abdominal pain. The clinical manifestations of food intolerance also include classic allergic symptoms such as anaphylaxis, allergic rhinitis,

atopic dermatitis, and urticaria. The role of food intolerance in conditions such as migraine headaches and allergic tension-fatigue syndrome is controversial. It is important to remember that the symptoms of food intolerance, especially gastrointestinal symptoms, can be mimicked by a variety of other conditions.

Leading institutions in Finland, China, England, and the USA have all recently published studies linking food intolerance/allergies to various symptoms and conditions including IBS. Evidence suggests significant reduction in IBS symptom severity in patients on elimination diets, provided that dietary elimination is based on foods against which the individual had raised IgG antibodies.

## III. PRINCIPLE OF THE TEST

Specific allergens are immobilized separately onto microtitre 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 MED** = Food Extract Coated (Med) Microwell Plates ..... 3 plates  
**DIL SPE 1X** = Serum Diluent (Green) ..... 1 x 56 ml  
**BUF WASH 66.67X** = Wash Buffer (concentrate) ..... 1 x 30 ml  
**CAL FOOD IgG** = Food IgG Calibrator ..... 1.0 ml  
**CRTL + IgG** = Food IgG Positive Control ..... 1.0 ml  
**CONJ ENZ IgG-HRP** = Food IgG-HRP Conjugate ..... 1 x 40 ml  
**SUBS A TMB** = Substrate Solution A (TMB) ..... 2 x 12 ml  
**SUBS B H2O2** = Substrate Solution B (hydrogen peroxide) ..... 2 x 12 ml  
**SOLN STOPPING** = Stopping Solution (1N H<sub>2</sub>SO<sub>4</sub>) ..... 1 x 20 ml

## V. WARNINGS AND PRECAUTIONS

### 1. Potential Biohazardous Material

The matrix 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<sub>2</sub>SO<sub>4</sub>. 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.

## VI. PREPARATION OF PATIENT SAMPLE

Dilute patient's serum 1:100 in Serum Diluent. Take 0.1 ml of patient serum and add to 10 ml of Serum Diluent.

## VII. REAGENT PREPARATION AND STORAGE

- Wash Buffer:** Wash the contents of the vial into a 2000 ml flask with distilled water and Q.S. to 2000 ml mark with distilled 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.
- Substrate Solution:** Mix Substrate Solution "A" and "B" in equal proportions 30 minutes before use. (*For example mix 5 ml each of "A" and "B" for each patient 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.

## 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 and 100 µl of Positive Control to well 1F.

- Place 100 µl of the diluted patient serum (**See Preparation of the Patient Sample – Section VI above**) into all the other wells. There should be 100 µl of liquid in all the wells.
- Cover the plates with parafilm or plastic wrap and incubate at room temperature (22-25°C) for 1 hour.
- 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.
- Add 100 µl of Food IgG-HRP Conjugate to all the wells.
- Incubate the plates for 30 minutes at room temperature (22-25°C).
- Wash the plates again as in step #4.
- Add 100 µl of Working Substrate mix to all the wells (see Reagent Preparation and Storage).
- Cover the plates and Incubate for 10 minutes at room temperature (22-25°C).
- Add 50 µl of Stopping Solution to all the wells. (Blue color in the wells will change to yellow).
- Set the microplate reader at 450 nm and read absorbance in all the wells.
- Plot the curve, using absorbance vs. the concentrations in the wells 1A -1E, on linear graph paper. A sample curve is given below. **Do not use sample for data calculation.**

| Well Position | Concentration | Absorbance |
|---------------|---------------|------------|
| 1A            | 0 U/ml        | 0.070      |
| 1B            | 50 U/ml       | 0.562      |
| 1C            | 100 U/ml      | 0.858      |
| 1D            | 200 U/ml      | 1.221      |
| 1E            | 400 U/ml      | 1.767      |
| 1F            | Pos. Cont.    | 1.408      |
| 1G            | Pat. Sample   | 0.280      |

## IX. 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. Well 1A           | < 0.2         |
| O.D. Well 1B           | > 1.2 x OD 1A |
| O.D. Well 1C           | > 1.2 x OD 1B |
| O.D. Well 1D           | > 1.2 x OD 1C |
| O.D. Well 1E           | > 1.2 x OD 1D |
| Concentration Positive | > 100 U/ml    |

## X. INTERPRETATION OF RESULTS









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

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

## XI. REFERENCES

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3. Eastham, E.J. and Walker, W.A. Adverse effects of milk formula ingestion on the gastrointestinal tract. *Gastroenterol.*, **76**, 366, 1979.
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5. All, M., et al. Serum concentration of allergy specific IgG antibodies in inhalant allergy: Effects of specific immunotherapy. *Amer. J. Clin. Pathol.*, **80**, 290, 1983.
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## XII. SYMBOLS

|   |                             |
|---|-----------------------------|
|  | Storage Temperature         |
|   | Lot Code                    |
|  | Expiration                  |
|  | Manufacturer                |
|   | Authorized Representative   |
|  | Caution, see instructions   |
|   | For in vitro diagnostic use |
|   | Catalog No.                 |

## XIII. ORDERING INFORMATION

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



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## 90 Foods Mediterranean (1 Patient) Microplate Map

|          | 1                   | 2                      | 3           | 4                   | 5                  | 6                       | 7                   | 8               | 9          | 10      | 11                | 12                 |
|----------|---------------------|------------------------|-------------|---------------------|--------------------|-------------------------|---------------------|-----------------|------------|---------|-------------------|--------------------|
| <b>A</b> | BLANK               | Apple                  | Broccoli    | Chard               | Codfish            | Garlic                  | Lettuce,<br>Iceberg | Mustard<br>Seed | Pear       | Rice    | Squashes          | Trout              |
| <b>B</b> | Calibrator 1        | Artichoke              | Butter      | Cheese<br>(Cottage) | Coffee             | Grape,<br>White/concord | Lemon               | Oat             | Pepper     | Rye     | Squid             | Tuna               |
| <b>C</b> | Calibrator 2        | Asparagus              | Cabbage     | Cheese<br>(Cured)   | Cola nut           | Grapefruit              | Lentils             | Olive           | Pinto bean | Salmon  | Strawberry        | Turkey             |
| <b>D</b> | Calibrator 3        | Avocado                | Cane sugar  | Chick<br>Peas       | Corn               | Green pea               | Lima bean           | Onion           | Pineapple  | Sardine | String bean       | Walnut,<br>black   |
| <b>E</b> | Calibrator 4        | Banana                 | Cantaloupe  | Chicken             | Cow's Milk         | Green pepper            | Lobster             | Orange          | Plum       | Shrimp  | Sunflower<br>seed | Wheat              |
| <b>F</b> | Positive<br>Control | Barley,<br>whole grain | Carrot      | Chocolate           | Cucumber           | Hake                    | Malt                | Parsley         | Pork       | Sole    | Sweet potato      | Yeast,<br>Baker's  |
| <b>G</b> | Almond              | Beef                   | Cauliflower | Cinnamon            | Egg,<br>white/yolk | Honey                   | Marjoram            | Peach           | Potato     | Soybean | Tea, black        | Yeast,<br>Brewer's |
| <b>H</b> | American<br>Cheese  | Beets                  | Celery      | Clam                | Eggplant           | Lamb                    | Mushroom            | Peanut          | Rabbit     | Spinach | Tomato            | Yogurt             |



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a tu lado desde 1987

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