

90 FOOD IgG SCREENING ELISA KIT

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

REF FIGG-90  3 x 96 test/kit

MEDITERRANEAN PANEL

INTENDED USE

The Food IgG Screening Elisa Test is for measuring the relative amount of food-specific IgG antibody to 90 foods 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. This kit does not provide information about IgE-mediated allergies.

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.

ASSAY PRINCIPLE

This kit is based on Elisa (Enzyme Linked Immuno Sorbent Assay) micro plate technology. It detects specific IgG antibody level of patients. 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. After terminating the reaction, the absorbance value is then measured at wave length 450nm. The absorbance value is directly proportional to (cubic polynomial linearity) the concentration of IgG antibody specific to a particular allergen.

MATERIALS PROVIDED

- 90 Foods Coated Microwells**
.....3 plates x 96 wells/plate
- Serum Diluent (Green)** consisting of carrier protein dissolved in Tris buffer
.....1 x 56 ml
- Foods IgG Calibrator** consisting of human serum to be used for creating standard curve
.....1 x 1.0 ml
- Foods IgG Positive Control** consisting of human serum to be used for ensuring the validity of the reagents
.....1 x 1.0 ml
- Wash Buffer (concentrate)** consisting of tween dissolved in phosphate buffer
.....1 x 30 ml
- Foods IgG-HRP Conjugate** consisting of goat anti-human IgG conjugated with horseradish peroxidase and carrier protein dissolved in Tris buffer
.....1 x 33 ml
- Substrate Solution A** consisting of tetramethylbenzidine (TMB) dissolved in citric acid buffer
.....2 x 12 ml
- Substrate Solution B** consisting of hydrogen peroxide dissolved in citric acid buffer
.....2 x 12 ml
- Stopping Solution (1N H₂SO₄)** consisting of 0.5 M sulfuric acid
.....1 x 20 ml

Note: This test kit performs as a complete system and do not combine reagents of other suppliers or kit components of different lots with this kit. Do not use components exceeding the expiry date.

MATERIALS REQUIRED BUT NOT PROVIDED

- Containers for preparation of wash buffer, substrate and diluting serum samples and foods IgG calibrator.
- Precision pipettes to deliver solution
- Parafilm
- Distilled or de-ionized (D.I.)water
- Full-automatic or semi-automatic microplate reader with 450nm, and capable of measuring 2.0 absorption unit.

SHELF LIFE AND STORAGE

Kit is stored at 2-8°C until stated expiration date. Do not freeze any kit component. The shelf life is 18 months under proper storage conditions. Do not use any kits beyond the stated expiration date.

SPECIMEN COLLECTION

Testing is performed on serum samples. The test requires approximately 0.1 ml of serum. Serum is collected according to standard practices and is stable for 8 hours at room temperature. Serum may be stored at 2-8°C for up to two days and may be frozen at or below -20°C for up to one year.

REAGENT PREPARATION AND STORAGE

Wash Buffer

Add 30ml of Wash Buffer into distilled or D. I. water and Q.S.to 2000ml as the final volume. Label it as Working Wash Buffer and store at 2-8°C. The Working Wash Buffer is stable for 6 months at 2-8°C.

Note: In some instances the Wash Buffer Concentrate (3:200) may develop crystals upon storage at 2-8°C. It is important that these crystals are completely re-dissolved before dilution of the Concentrate. This can be accomplished by warming the Concentrate to 37°C in a water bath with occasional mixing.

Substrate Solution

Mix Substrate Solution A and B in equal proportions for 30 minutes before use. For example mix 5 ml each of A and B for each plate to be used.

Note: 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.

ASSAY PROCEDURE

1. Bring all the test kit reagents to room temperature (20-28°C) before use.
2. **PREPARATION FOR A SINGLE 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 IgG 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

3. Add 100 µl of Serum Diluent to wells 1A for the blank wells and 100 µl of Positive Control to wells 1F.
4. Add 10 ml of Serum Diluent to 0.1 ml of patient sample and mix them up.
Note: Dilute Food IgG Calibrator as step 3 described. No need to dilute Foods IgG Positive Control.
5. Place 100µl of the diluted patient serum into all the other wells. There should be 100 µl of liquid in all the wells.
6. Cover the plates with parafilm or plastic wrap and incubate at room temperature (20-28°C) for 60±2 minutes.
7. After incubation for 60±2 minutes, wash all the microwells three times with 300 µl working wash buffer manually or by an automated washer. The microwells should be filled to the full by working wash buffer. Following the wash step, drain the microwells.
8. Add 100 µl of Foods IgG-HRP Conjugate to all the wells. Incubate the plates for 30±2 minutes at room temperature (20-28°C).
9. Wash the plate 3 times as in step 8.
10. Add 100 µl of Working Substrate mix to all the wells. Cover the plates and Incubate for 10±2 minutes at room temperature (20-28°C).
11. Add 50 µl of Stopping Solution to all the wells, and clean the residual solution at the bottom of the microplate carefully with soft gauze/paper towel(Blue color in the wells will change to yellow).
12. Set the microplate reader at 450 nm and read absorbance in all the wells.

ADVICE FOR ASSAY PROCEDURES

1. Close adherence to the wash procedure will assure optimal performance. Do not shorten or lengthen stated

washing times since this may result in high background signal.

2. Except substituting the patient serum in blank wells with Serum Diluent, other assay procedures described should be strictly followed.
3. Use Positive Control to monitor reagent stability and correct assay performance.
4. Use Food IgG Calibrator for creating standard curve.

CALCULATION OF RESULTS

In order to eliminate the effects of washing variation, instrument variability, etc. specimen values are normalized according to the following cubicpolynomial formula:

$$Y=AX^3+BX^2+CX+D$$

Note: X represents standardization activity (U/ml) of serum sample or positive control.

Y represents absorbance of serum sample or positive control.

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

INTERPRETATION OF RESULTS

The absorbance readings after extrapolation as U/ml should be interpreted as for each allergen or extract. The grading standard for negative results is referring to the test results from normal serum. In most cases, a result with the values within range <50U/ml are negative, but sometimes it may be weak positive for some individual normal serum.

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

LIMITATIONS

This test kit includes many kinds of food allergens. Both the positive and negative results can only be related to the allergens coated in the microwells and it cannot prove that patients are or are not allergic to the other allergens.

PERFORMANCES

Linearity Range

The range of the standard curve of this kit is 0~400U/ml. Specimens with higher IgG concentrations should be diluted and retested to determine the exact IgG content.

Sensitivity

The sensitivity of this kit is no more than 50 U/ml.

Specificity

Hemaglobulin, triglycerides, IgE, IgM, and IgA all have no influence to the test results of this kit.

Reproducibility

Intra-lot variable coefficient (N=40): 11%; inter-lot variable coefficient (N=44): 15%.

Clinical Trials Results

The clinical trials show that general people may also have

certain food specific IgG antibody, therefore, clinical diagnosis could not be given by referring to any test result. The test results must always be correlated to the clinical condition of the patient.

WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only.
2. Close adherence to the test procedure will assure optimal performance. Do not shorten or lengthen stated incubation times since this may result in poor assay performance.
3. Some reagents contain sodium azide which may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.
4. Stopping Solution consists of 1N H₂SO₄. 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. Rinse immediately with plenty of water if any contact occurs.
5. 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 agents.

REFERENCES

1. Anderson, C.M. and Burke, V. Pediatric Gastroenterology, Oxford: Blackwell, 1975.
2. Back, S.A., Lee, W.Y., Remigio, L.K. and May, C.C. Studies of hypersensitivity reactions to foods in infants and children. J. Clin. Allergy Immunol., 62, 327, 1978.
3. Eastham, E.J. and Walker, W.A. Adverse effects of milk formula ingestion on the gastrointestinal tract. Gastroenterol., 76, 366, 1979.
4. Kuitunen, P., Visakorpi, K. J., Savilahti, E. and Pelkonen, P. Malabsorption syndrome with intolerance: Clinical findings and course in 54 cases. Arch. Dis. Child., 54, 351, 1975.
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.
6. Farrel, M. Food allergy, in Manual of Allergy and Immunology, ed: Lawlor, G.J. and Fischer, T.J., 1982.



Meridian Healthcare srl

Via Caronda, 446/SC. A , 95129 Catania , Italy
Tel: +39 095 725 68 69



90 FOODS SPLIT MICROPLATE MAP

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

				8°C 2°C	
Lot-number	European conformity	Sufficient For <n> tests	For in vitro Diagnostic use	Temperature Limit	Use before
Catalogue Number	Consult instructions for use	Refer accompanying documents	Do not use when Package is damaged	Do not Re-use	Manufactured by



Meridian Healthcare srl

Via Caronda, 446/SC. A , 95129 Catania , Italy
Tel: +39 095 725 68 69

