HOB® Allergy Line

HOB-LIA-Food Allergen Panel

Specific IgE Screening Test Kit for the Detection of Food Allergens

REF MB00119

Revision 03, 09/10/2015

KEY TO SYMBOLS USED

REF List Number	Expiration Date
IVD For *In Vitro Diagnostic* Use	LOT Lot Number
8°C 2°C Store at 2-8°C	Manufacturer
Consult Instructions to Use	EC REP Authorized Representative

See **MATERIALS PROVIDED** & **MATERIALS REQUIRED BUT NOT PROVIDED** for a full explanation of symbols used in reagent component naming.

CE

HOB Biotech Group
C6 Building, 218 Xinghu Rd., Suzhou Industrial Park,
Jiangsu Province, 215123, PR China.
Tel: +86 (512) 69561996
Fax: +86 (512) 62956652

EC REP **Eurohob (UK) Co.Ltd**
59 Tycehurst Hill, Lougton, Essex, IG10 1BZ UK
Tel: +44(7786910086)

HOB-LIA-Food Allergen Panel

Specific IgE Screening Test Kit for the Detection of Food Allergens

REF MB00119

SPECIFICATION

SIgE-20 Allergens + CCD, FD-1, 20 Tests

INTENDED USE

Food Allergen Panel 1 is for the semi-quantitative measurement of the relative amount of specific IgE antibodies to 20 food allergens as well as CCD in human serum.

SUMMARY&EXPLANATION OF TEST

Immunoglobulin class E was discovered in 1966 by the Japanese scientist couple Teruko and Kimishige Ishizaka. It plays an essential role in Type I hypersensitivity. There are over 15% people suffering from Type I allergic reaction in industrialized country. In allergic patients, they will have multiple syndromes, such as dermatitis, rhinitis, hay fever, asthma, edema, diarrhea, and pruritus. This assay provides visual detection of allergen-specific IgE in human serum. The detection of increased amounts of circulating allergen specific IgE, when interpreted in context with allergic history and physical examination, can contribute to the diagnosis and assessment of IgE-mediated allergic diseases.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The assay is a two-step immunoassay to determine the presence of allergen-specific IgE in human serum. In the first step, the allergen-specific IgE antibodies in the sample bind to the corresponding allergens which are coated on nitrocellulose membranes. After washing, alkaline phosphatase-labeled anti-human IgE antibodies are added in the second step. The strip is then washed again to remove any unbound AP-anti-IgE antibody. After addition of the substrate solution, a visual color change will be observed which indicates the existence of specific IgE antibodies against respective allergens. The intensity of color is proportional to the amount of specific IgE antibodies in the serum.

MATERIALS PROVIDED

- ***Test Strips* coated with** CCD and 20 food allergens, ready to use. STRIP
  ……..1 X 20 strips for 20T;
- ***Sample Diluent*** green liquid, consisting of goat serum in Tris buffer, ready to use. DIL
  ……..1 X 50 mL for 20T;
- ***Wash Buffer10X concentrate*** transparent liquid, consisting of a 10X concentrate of Tween 20, dilute with distilled or de-ionized (D.I.) water before use. BUF WASH 10x
  ……..1 X 30 mL for 20T;
- ***IgE Conjugate Solution*** red liquid, consisting of anti-human-IgE AP conjugate, ready to use.
  ……..1 X 30 mL for 20T; CONJ
- ***Substrate Solution*** light yellow liquid, consisting of NBT/CBIP, ready to use. SUBS
  ……..1 X 30 mL for 20T;
- ***Scoring Sheet***
  ……1pc for 20T;
- ***Incubation Tray***
  ……2pcs for 20T;
- ***Package Insert***
  ……1pc for 20T;

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or de-ionized water H_2O DIST
- Shaker
- Timer
- Pipette (200-1000 µL)
- Absorbent paper
- Latex gloves

Illustration of Food Panel allergen

Food Panel		
No.	**CODE**	**ALLERGEN**
1	C	Control Line
2	CCD	Cross-reactive carbohydrate Determinant
3	F84	Kiwi
4	F31	Carrot
5	F85	Celery
6	F25	Tomato
7	F49	Apple
8	F95	Peach

9	F92	Banana
10	F27	Beef
11	F23	Crab
12	F3	Codfish
13	F202	Cashew nut
14	F20	Almond
15	F17	Hazelnut
16	F4	Wheat
17	F10	Sesame
18	F5	Rye flour
19	F14	Soy beans
20	F13	Peanut
21	F2	Milk
22	F1	Egg white

SPECIMEN COLLECTION

Use fresh patient specimens only or freeze samples at -20°C. Freeze samples only one time prior to use. Do not use 56°C heat inactivated samples.

Sera with interfering substances (haemolysis, blood lipid or jaundice), of which the concentration is 3 times higher than normal value, are not recommended to be tested by this assay.

Serum, which is collected according to standard procedure, is stable for 72 hours at 2-8°C and may be frozen at or below -20°C for longer time. Do not freeze and thaw the serum frequently.

SAMPLE PREPARATION

Dilute serum samples 1:1 with Sample Diluent.
For example, dilute 0.5mL of sample with 0.5mL Sample Diluent.

REAGENT PREPARATION AND STORAGE

Attention!

- Allow the test kit and all its components to reach room temperature before use.
- Used bottles should be closed carefully and stored at 2-8°C.
- Unused strips should be sealed into the aluminum pouches together with desiccant.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.

Wash Buffer 10X Concentrate

Dilute one part with nine parts distilled or de-ionized (D.I.) water. Diluted wash buffer is stable for 6 weeks stored at 2-8°C. Any crystallized salt inside the bottle must be resolved before use.

RCNS	H2O

ASSAY PROCEDURE

Attention!

- Do not let test strip dry during the incubation steps.
- Do not touch test strip with fingers, use tweezers.
- Remove diluted samples completely after incubation of test strip to avoid cross contamination.
- Please shake test strips at room temperature with gentle agitation during all incubation steps.
- All details are valid for one strip or patient sample.

1. Put test trip into the incubation tray, each incubation tray carries one strip. Add 1 mL of Sample Diluent into each incubation tray, and incubate the test strip for 5 minutes at room temperature with gentle agitation, and then remove it.
2. Pipette 1 mL of diluted patient sample and add it into the incubation tray, incubate for 60 minutes at room temperature with gentle agitation.
3. Remove diluted samples completely. Wash test strip 3 times using 1 mL Wash Buffer for 5 minutes with gentle agitation. Remove Wash Buffer after every washing step.
4. Pipette 1 mL Conjugate Solution and incubate for 30 minutes at room temperature with gentle agitation. Remove Conjugate.
5. Repeat washing steps of step 3.
6. Add 1 mL Substrate Solution and incubate for 15 minutes at room temperature with gentle agitation.
7. Remove Substrate. Wash with 1 mL distilled or de-ionized (D.I.) water for 1 minute at room temperature with gentle agitation. Repeat this wash step for another 2 times.
8. Dry test strip, and stick it onto the Scoring Sheet to record the test results.

QUALITY CONTROL PROCEDURES

A control line is provided on each test strip. The test result is valid if a visible control line is presented.

INTERPRETATION OF RESULTS

Score the test results according to the coloring intensity of the strip as Class 0-6. The test result is negative for a given allergen if no band is to be recognized, designated as Class 0. The test result is positive for a given allergen if a band exhibits a staining, and based on different coloring intensities, designated as Class 1-6.

IU/mL	Class	Allergen-specific IgE content
<0.35	0	None or hardly any found
0.35-0.69	1	Low
0.7-3.49	2	Increased
3.5-17.49	3	Significantly increased
17.5-49.9	4	High
50-100	5	Very high
>100	6	Extremely high

A positive result indicates the presence of circulating IgE to the test allergen, suggestive of IgE-mediated hypersensitivity to the allergen and caution should be take in further investigative procedures. However, a positive result does not necessarily indicate clinical sensitivity to the test allergen since some individuals may elicit positive results in the absence of clinical allergy.

A negative result indicates the absence of circulating IgE to the test allergen, suggesting that symptoms may be due to other causes. However, a negative result does not exclude clinical sensitivity since some individuals may elicit negative test results in the presence of clinical allergy. Allergic hypersensitivity reactions to altered, (e.g. by industrial processing, cooking or digestion) or related food allergens may be also be present, therefore caution should be taken in further investigative procedures.

TEST LIMITATIONS

1. A positive result must be used in associated with clinical evaluation and diagnostic procedures. The values obtained from this assay are intended to be an aid for IgE-mediated allergic disease diagnosis only.
2. Related allergens contain common allergic determinants capable of cross-reacting and eliciting positive test results.
3. Immunosuppressive drug therapy (anti-inflammatory corticosteroids) as well as physiological and genetic conditions may affect the levels of allergen-specific IgE in the circulation.
4. Allergen desensitization therapy may inhibit the test response to those allergens included in the desensitization treatment as well as to other allergens which are commonly cross-reactive to those included in the desensitization therapy.

PERFORMANCE CHARACTERISTICS

- **Precision (within-run)**

Class	CV%
1	15.9
2	7.89
3	8.04
4	6.96
5	5.08
6	2.57

- **Precision (Between--run)**

Class	CV%
1	18.5
2	11.4
3	8.0
4	6.3
5	6.6
6	4.0

- **Interference**

  Using hemolytic, lipemic, icteric serum may lead to erroneous results.

  Serum must meet the following criterion:

  Triglyceride: ≤30mg/mL

  Hemoglobin: ≤5mg/mL

  Bilirubin: ≤0.2mg/mL

- **Methods Comparison:**

ALLERGEN	CODE	SENSITIVITY [%]	SPECIFICITY [%]
Egg white	F1	90	82
Milk	F2	80	90
Wheat	F4	88	81
Sesame	F10	80	92
Peanut	F13	88	94
Soy beans	F14	85	92
Carrot	F31	90	95
Cross-reactive carbohydrate determinant	CCD	98	100

All results were calculated in comparison with Phadia Immunocap.

WARNINGS AND PRECAUTIONS

- **IVD** For In Vitro Diagnostic Use
- Safety and handling precautions
  1. Do not use components exceeding the expiration date.
  2. Do not combine reagents of other suppliers or kit components with this kit.
  3. Do not interchange caps of kit components.
  4. Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens should be handled as potentially infectious. Wear protective clothing and disposable gloves according to Good Laboratory Practices.
  5. Substrate Solution is light sensitive reagent and avoids exposing this reagent to illumination. Do not use Substrate Solution if it gets turbid or blue.
- Shelf life and storage instructions

  

  The shelf life of HOB Food Allergen Panel 1 Kit is 18 months under storage conditions of 2-8° C. Do not freeze any components.
- Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

BIIBLIOGRAPHY

1. Ishizaka K, Ishizaka T, Hornbrook MM. Physico-chemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. J. Immunol. 97 (1), 1966
2. Gould H et al. The biology of IgE and the basis of allergic disease. Annurev.Immunol.21, 2003
3. Adkinson, Franklin, N. In vitro Tests for IgE in the Practice of Allergy. Insights in Allergy Vol. 1, December 1986.
4. Atkinson, N.D. and Katz, D.H.,Quidel's Novel Allergy Diagnostic Test. Presented at XII International Congress of Allergology and Clinical Immunology. Washington, D.C., October 15-20, 1985.
5. Novey, H.S., Iman, A.A., Orgel, H.A., Meltzer, E.O., Kemp, J.P., Welch, M.J., Klein, G.L., Ziering, R.W. and Miller, M.F. Multi Allergen Dipstick Screening Test for Specific Serum IgE (sIgE). Presented at American Academy of Allergy and Immunology, February 20-24, 1987.
6. Sampson, H.A., Buckley, R.H. and Metcalfe, D.D., Food Allergy, JAMA: 20, 1987, pp. 2886-2890.
7. Golbert, T.M., in Allergic Diseases: Diagnosis and Management, Roy Patterson (editor), 3rd edition, 1985, pp. 459-475.
8. Ishizaka, K. and Ishizada, T. Identification of –E antibodies as a carrier of reaginic activity. J. Immunol. 99: 1187, 1967.
9. Johansson, S.G.O. and Bennich, H.A. new class of immunoglobulin in human serum. Immunology 14:265, 1968.
10. Austen, K.F. in Harrison's Principles of Internal Medicine, 10th edition, 1983, p.372.
11. McFadden, E.R., Jr., and Austen, K.F. IN Harrison's Principles of Internal Medicine, 10th edition, 1983, p. 1512.