Foresight®

Allergen Test Kit

Package Insert

REF I031-1011	English

An immunoblotting assay for the qualitative determination of circulating Allergen Specific Immunoglobulin E (IgE) in human serum. For professional in vitro diagnostic use only.

INTENDED USE

The Allergen Test Kit is an immunoblotting assay for the qualitative determination of circulating Allergen Specific Immunoglobulin E (IgE) in human serum.

SUMMARY

A national survey in the United States found that 56.4% of all U.S. citizens test positive to one or more allergens[1]. A steady increase in the prevalence of allergic diseases globally has occurred with about 30-40% of the world population now being affected by one or more allergic conditions [2]. Immunoglobulin E (IgE) is specifically important to food allergies that is part of Type I hypersensitivity. Ordinarily IgE is produced to fight infection caused by parasites. This molecule is also produced in harmless things such as pollen, dust and foods that may cause allergic diseases like asthma, allergic rhinitis and food allergy.

PRINCIPLE

The Allergen Test Kit is an immunoblotting assay for the qualitative detection of IgE antibody to Allergen in human serum. The surface of nitrocellulose membranes of test trips are coated with specific allergens. During testing, the specimen is added to the test strip and then incubated. If the specimen contains allergen-specific IgE antibodies, it can react with the allergens and bind to the nitrocellulose membrane of the strip. Unbound material is removed by washing. Detector antibodies (Anti-human IgE antibody coupled with Biotin) are then added to the strip and incubated. The Detector antibodies will bind to the respective specific IgE which are bound to the strip in the first incubation. Unbounded Detector antibodies are removed by washing. Next, Streptavidin conjugated with Alkaline Phosphatase is added to the test strip and incubated. This will bind to the Biotin which are bound to the strip in the second incubation. Unbounded Streptavidin conjugate is removed by washing. Then substrate is added and incubated, which will cause a specific enzymatic color reaction of the Alkaline Phosphatase.
The color intensity which corresponds to the amount of Allergen specific antibody is interpreted by comparison with a color chart.
To serve as a procedure control, a colored line will always appear in the control line region which indicates the correct procedure.

WARINGS AND PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not mix reagents from other kits with different lot numbers.
- Avoid cross contamination between reagents to ensure valid test results.
- Human specimens should be considered potentially hazardous and handled using established good laboratory working practices.
- Some components of this kit contain ProClin™ 300. Avoid any contact with skin or eyes.
- Conjugate contains Methylisothiazolone and Bromonitriidodxane in sub toxic concentrations as preservatives.
- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection while handling kit reagents and specimens. Wash hands thoroughly when finished.
- Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not mouth pipette.
- Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
- Handle and dispose all specimens and materials used to perform the test as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all the procedures and follow the standard procedures for proper disposal of specimens.
- Observe Good Laboratory Practices when handling chemicals and potentially infectious material. Discard all contaminated material, specimens and reagents of human origin after proper decontamination and by following local, state and federal regulations.
- Neutralized acids and other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to a 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

STORAGE AND STABILITY

- Unopened test kits should be stored at 2-8°C upon receipt. All unopened reagents are stable through the expiration date printed on the box if stored between 2-8°C. Once opened, all reagents are stable for up to 1 month after the first opening date if stored between 2-8°C. Return reagents to 2-8°C immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution in a water bath at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- Do not expose reagents, especially the Substrate, to strong light or hypochlorite fumes during storage or incubation steps. If the substrate becomes colored, it will no longer be suitable for use.

SPECIMEN COLLECTION PREPARATION

- This kit can be performed using only human serum specimens. The blood samples should be acquired using venipuncture and separating the serum out after coagulation (30-40 minutes) by centrifugation for 10 minutes at 4000g.
- Separate serum from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Specimens with extensive particulates should be clarified by centrifugation prior to use. Do not use specimens with fibrin particles or contaminated with microbial growth.
- Do not leave specimens at room temperature for prolonged periods. Serum specimens may be stored at 2-8°C for up to 7 days prior to assaying. For long term storage, specimens should be kept frozen at temperatures below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

REAGENTS AND COMPONENTS

Materials Provided

No.	Reagent	Component Description	Quantity
	Test Strips	Test Strips in plastic reaction troughs, nitrocellulose membranes coated with allergen material	10
1	Concentrated Wash Buffer (25x)	Tris / NaCl, contains 0.1% Proclin™ 300, yields 1 X 500 mL washing buffer, pH = 7.5	1 x 20 mL
2	Detector Antibody	Bottle with white cap, ready for use. With Biotin conjugated Goat Anti-Human IgE Antibodies	1 x 4 mL
3	Conjugate	Bottle with red cap, ready for use. Streptavidin conjugated with Alkaline Phosphatase contains 0.02% Methylisothiazolone and 0.02% Bromonitriidodxane	1 x 4 mL
4	Substrate	Bottle with black cap, ready for use. BCIP / NBT (Bromochloroindolyl Phosphate/ Nitro Blue Tetrazolium)	1 x 4 mL
	Color Chart		1
	Package Insert		1
	Quick Start Guide		1

NOTE: Use only the components with the same lot number.

Test Strip Configuration

Location	Abbreviation	Test Items
1	Control line	To check if the test has been carried out properly.
2	D1/D2	Der.pteronyssinus, Der.farinae,
3	W1/W6	Short ragweed, Mugwort
4	E1/E5	Cat dander, Dog dander
5	Spacing line (Light red color)	
6	I6	Cockroach
7	M1/M2/M3/M6	Penicillium Chrysogenum, Cladosporium Herbarum, Aspergillus Fumigatus, Alternaria Alternata
8	W22	Japanese Hop
9	T7/T8/T11/T12/T14	White Oak, Elm, Sycamore, Willow, Cottonwood
10	Spacing line (Dark red color)	
11	F1	Egg white
12	F2	Cow's milk
13	F3/F24/F23	Fish, Shrimp, Crab
14	F27/F88	Beef, Mutton
15	Spacing line (Dark red color)	
16	F202/F13/F14	Cashew nut, Peanut, Soybean
17	F91	Mango
18	F4	Wheat
19	Total IgE	Total IgE

Materials Required But Not Provided

- Freshly distilled or deionized water
- Graduated cylinders for wash buffer dilution
- 500 mL laboratory wash bottle
- Shaker
- Incubation box (for incubation in the dark)
- Vortex mixer
- Timer
- Hairdryer
- Disposable gloves
- Disposable reagent reservoirs

DIRECTIONS FOR USE

Allow reagents and specimens to reach room temperature (18-25°C) prior to testing. The reagents must be thoroughly mixed before use. The reproducibility of the results is strongly dependent on the accuracy of pipetting, maintenance of the incubation times and temperature, as well as the uniformity of washing of the test strips. The procedure must be strictly followed. Assay must proceed to completion within time limits.

NOTE:

1. Make sure the reaction trough is completely covered by solutions during each of the dampening, incubation and washing

steps.

2. The substrate incubation must be carried out in the dark to avoid auto-coloration of the substrate. It is strongly recommended to incubate the Test Strips in an incubation box during each incubation process.

Step	Detailed Procedure
1	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Dilute 1 volume of Concentrated Wash Buffer (25x) with 24 volumes of freshly distilled or deionized water in a wash bottle. Mix well before use.
2	• Add 250 µL the Working Wash Buffer into the reaction trough; wet the reaction trough by shaking it (on a shaker) at room temperature (18–25°C) for 5 minutes. Remove the Working Wash Buffer and wipe the excess water on the plastic surface using absorbent tissue.
3	• **First Incubation:** Add 250 µL of serum specimens into the reaction troughs then incubate on the shaker at room temperature (18–25°C) for 45 minutes. Remove the serum specimens after incubation. **NOTE:** Discard the serum specimen carefully and avoid cross contamination of the incubation trough.
4	• Wash test strips 6 times with Working Wash Buffer while holding the reaction trough diagonally. Shake the solution in the trough manually for about 10 seconds and then remove the Working Wash Buffer. Wipe the excess solution on the plastic surface using absorbent tissue. **NOTE:** Complete washing is important. Shake the Working Wash Buffer in the trough for several seconds before pouring it out to ensure effective washing.
5	• **Second Incubation:** Add 5 drops (250 µL) of the Detector Antibody then incubate on the shaker at room temperature (18–25°C) for 45 minutes. Remove excess Detector Antibody after incubation.
6	• Repeat Step 4.
7	• **Third Incubation:** Add 5 drops (250 µL) of Conjugate then incubate on the shaker at room temperature (18–25°C) for 20 minutes. Remove excess Conjugate after incubation.
8	• Repeat Step 4.
9	• **Fourth Incubation:** Add 5 drops (250 µL) of Substrate then incubate (in the dark) on the shaker at room temperature (18–25°C) for 20 minutes in the dark. Remove excess Substrate after incubation. **NOTE:** The substrate incubation must be carried out in the dark to avoid auto-coloration of the substrate.
10	• Rinse the strip under flowing water to stop the substrate reaction. Wipe the excess water on the plastic surface using absorbent tissue.
11	• Dry the strip in the air or by using a conventional hair dryer which will speed up the drying process. The blue-purple color of the background disappears as the test strip dries. **NOTE:** The Test Strip should be dried completely before reading results.
12	• Compare the strip with the color chart. A qualitative evaluation of those lines is possible by visual comparison of the color intensity of each line on the strip with the color chart. **NOTE:** The control line should appear now, which is used to check if the test has been carried out properly.

INTERPRETATION OF RESULTS

Allergen-Specific IgE

Class	Color Intensity	Interpretation
-	No colored line appears	Negative
+	Faint colored line	Low concentration of Allergen Specific IgE Antibody
++	Distinct colored line	Medium concentration of Allergen Specific IgE Antibody
+++	Strong colored line	High concentration of Allergen Specific IgE Antibody

1. A negative result indicates that the patient is non-atopic.
2. A positive result indicates that the patient may be atopic to this allergen.
3. The higher concentration of Allergen-Specific IgE Antibody is, the higher degree the patient is suffering the atopic.

Total IgE

Class	Color Intensity	Interpretation
-	No colored line appears	The concentration of Total IgE is less than the detection limit
+	Faint colored line	Low concentration of Total IgE antibody
++	Distinct colored line	
+++	Strong colored line	High concentration of Total IgE antibody

1. Result +++ is positive which indicates the patient may be atopic.
2. The Total IgE test of this kit cannot replace the single, quantitative ELISA test system.

NOTE: The test results are considered invalid if the below validation requirements are not met.

VALIDATION REQUIREMENTS AND QUALITY CONTROL

A colored line appearing in the control line region is considered as an internal procedural control. It confirms correct procedural technique. The test should be considered as invalid if the color intensity of control line is below "++" when compared with the color chart. Check the expiration date of the test kit and make sure that all reagents have not been opened for more than 1 month. Review the procedure to make sure that the test procedure has been followed properly and repeat the test with a new test strip. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

LIMITATIONS

1. The Allergen Test Kit is used for determination of circulating Allergen Specific Immunoglobulin E (IgE) in human serum. Diagnosis should not be established based on a single test result. Further testing should be performed in assessing clinical status.
2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
3. The patients with positive results from Allergen Test Kit may not have related clinical symptoms if they are only during a sensitization period without clinical relevance.

PERFORMANCE CHARACTERISTICS

1. Sensitivity: 0.35 IU / mL
2. Specificity: the rate of the cross-reactivity of the human IgA, IgG and IgM, etc. <0.021%
3. Precision: Intra to repetitive >99.9%

BIBLIOGRAPHY

1. Arbes SJ et al. Prevalences of positive skin test responses to 10 common allergens in the U.S. population: Results from the Third National Health and Nutrition Examination Survey: J Allergy Clin Immunol. 2005; 116: 377-383
2. WAO (World Allergy Organization) White book 2011.

Index of Symbols

	Consult instructions for use		Tests per kit		Manufacturer
IVD	For *in vitro* diagnostic use only		Use by	Allergen	Allergen
	Store between 2-8°C	EC REP	Authorized Representative	Test Strip	Test Strip
Wash Buffer 25x	Wash Buffer (25x)	REF	Catalog #	Conjugate	Conjugate
Substrate	Substrate	Detector Antibody	Detector Antibody	Package Insert	Package Insert
Quick Start Guide	Quick Start Guide	Color Chart	Color Chart		

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