BioLINE Allergy Reagent Kit

(Immunoblotting)

REF

MB00276; MIX-20, E; 24 Tests/kit

INTENDED USE

BioLINE Allergy Reagent Kit is an in vitro semi quantitative assay for the determination of allergy specific IgE to multiple allergens.

Allergens include Dermatophagoides pteronyssinus/Cat dander/Dog dander /Egg white/Milk/Cashew nut/Pineapple/Crab/Shrimp/Beef/Scallop/Mango /House dust mite/Cockroach, German /Penicillium chrysogenum/Cladosporium herbarum/Aspergillus fumigatus /Alternaria alternata / London plane /Willow/Cottonwood/Oak /Mulberry /Elm/Common ragweed /Common pigweed /Mugwort.

It is intended for in vitro diagnostic use as an aid in the clinical diagnosis of IgE mediated allergic disorders in conjunction with other clinical findings, and is to be used in clinical laboratories for professional use.

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.

PRINCIPLES OF THE PROCEDURE

The kit adopts immunoblotting to detect total IgE antibody and allergen specific IgE antibody in human serum. IgE antibodies in the sample bind to mouse anti human IgE antibody and the corresponding allergens which are coated on nitrocellulose membranes to make the IgE antibody in serum combined with the corresponding reaction zone on the test strip. After washing, biotinylated mouse anti human IgE antibodies are added for incubation. After washing again, alkaline phosphatase-labeled Streptavidin are added to form mouse anti human IgE antibody-IgE antibody-biotinylated mouse anti human IgE antibody alkaline phosphatase-labeled streptavidin complex or allergen-specific IgE antibody -biotinylated mouse anti human IgE antibody alkaline phosphatase-labeled streptavidin complex.

The strip is then washed again to remove any unbound SA-AP. After addition of the substrate solution for incubation, the alkaline phosphatase fixed on the membrane strip by the above complex catalyzes the substrate reaction. Visual blue-purple color change will be observed which indicates the existence of total IgE antibody or specific IgE antibodies against respective allergens.

After the reaction, the reaction was terminated with purified water, and the gray value of the reaction area was read after drying. The gray value is proportional to IgE amount.

WARNINGS AND PRECAUTIONS

- 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.
- For professional in vitro diagnostic use only.
- For single use only. Do not re-use.
- The instruments used shall be routinely checked to ensure correct operation within their functional range
- Do not use reagents beyond the expiration dates.
- The kit contains human sourced materials. All recommended precautions for the handling of blood derivatives should be taken. Please refer to the OSHA Standard on Blood borne Pathogens or Biosafety Level [10] or other appropriate biosafety practices. [11,12]
- 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.
- Liquid waste and solid waste should also be

handled in accordance with the appropriate biosafety practices same as stated above.

Precautions

- The solutions contain ProClin300 ≤0.1% as preservative and surfactant and may cause an allergic skin reaction by skin contact. Avoid contact with skin. Wear protective gloves, protective clothing and take eye protection.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

MATERIALS PROVIDED

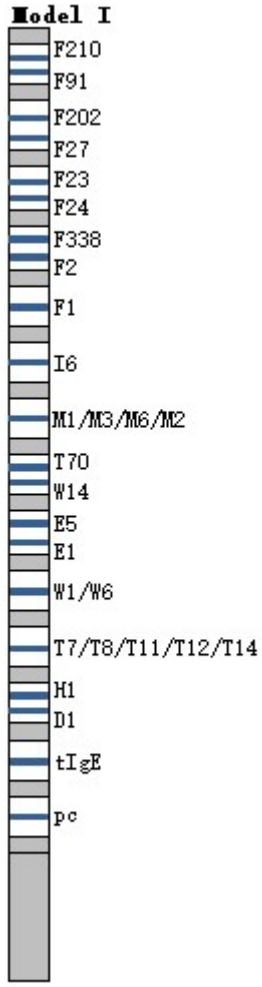
- ***Strips*** coated with the corresponding allergens on nitrocellulose membranes, ready to use.
  ……..1 X 24 strips for 24T; STRIP
- ***Sample Diluent*** 0.5% BSA in 0.05M Tris buffer, ready to use.
  ……..1 X 60 mL for 24T; DIL
- ***Biotinylated Anti-IgE antibodies*** consisting of 0.2μg/mL~0.8μg/mL biotinylated anti-human-IgE in 0.05M Tris buffer, ready to use.
  ……..1 X 40 mL for 24T; Anti-IgE | BIOT
- ***Streptavidin Conjugate*** consisting of 1:2000~1:8000 streptavidin AP conjugate in 0.05M MES buffer, ready to use.
  ……..1 X 40 mL for 24T; CONJ
- ***Substrate*** light yellow liquid, consisting of NBT/CBIP, ready to use.
  ……..1 X40 mL for 24T; SUBS
- ***Wash Buffer concentrate*** transparent liquid, consisting of a 10X concentrate in 0.05M Tris buffer , pH7.5±0.2.
  ……..1 X 50 mL for 24T; BUF | WASH | 10x
- ***Scoring Sheet***
  ……1pc for 24T;
- ***Incubation Tray***
  ……3pcs for 24T;

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 Panel allergen and test strip

No.	CODE	ALLERGEN
1	D1	Dermatophagoides pteronyssinus
2	E1	Cat dander
3	E5	Dog dander
4	F1	Egg white
5	F2	milk
6	F202	Cashew nut
7	F210	Pineapple
8	F23	Crab
9	F24	Shrimp
10	F27	Beef
11	F338	Scallop
12	F91	Mango
13	H1	House dust mite
14	I6	Cockroach, German
15	M1	Penicillium chrysogenum
16	M2	Cladosporium herbarum
17	M3	Aspergillus fumigatus
18	M6	Alternaria alternata
19	T11	London plane
20	T12	Willow
21	T14	Cottonwood
22	T7	Oak
23	T70	Mulberry
24	T8	Elm
25	tIgE	Total IgE
26	W1	Common ragweed
27	W14	Common pigweed
28	W6	Mugwort



REAGENT PREPARATION AND STORAGE

- Store the kit at 2-8 °C. The shelf life of the unopened kit is 18 months. Do not freeze any components.
- Vial opened reagents could be used for 28 successive days stored at 2-8°C. Used vials should be closed carefully and stored at 2-8°C.
- Do not use components beyond the expiration date.
- Do not combine reagents of other suppliers or kit components with this kit.
- Substrate Solution is light sensitive reagent and avoids exposing this reagent to illumination. Do not use Substrate Solution if it gets turbid or blue.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- Do not interchange caps of kit components.
- Do not use reagents and test strips with broken package.

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

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Serum from venous only can be used.
- Serum which is collected according to standard procedure is stable for 2 days at 2-8°C and may be frozen at or below -20°C for longer time.
- Before testing, the frozen samples shall be thawed at room temperature (20-25℃), gently reversed and mixed evenly. Avoid repeated freezing and thawing.
- Do not use 56°C heat inactivated samples.
- Sera with interfering substances (hemolysis, blood lipid or jaundice) are not recommended to be tested by this assay.
- The samples don't need to be diluted, ready to use.

ASSAY PROCEDURE

Reagent Preparation

Sample Diluent	ready to use.
Biotinylated Anti-IgE antibodies	ready to use.
Streptavidin Conjugate Solution	ready to use.
Substrate Solution	ready to use.

Wash Buffer 10X concentrate

Dilute one part with nine parts distilled or de-ionized (D.I.) water. Diluted wash buffer is stable for 1 weeks stored at room temperature.

ASSAY PROCEDURE

- Please shake test strips at room temperature with gentle agitation during all incubation steps. If the temperature is too high or too low, the results will not be within the quality control range.
- Washing steps are very important. Incorrect washing will lead to wrong results.
- In order to avoid cross contamination and false positive results, it is recommended to use the correct suction method.

Manual operation

1. Allow the test kit and all its components to reach room temperature before use. (no less than 30 minutes is recommended)
2. Put test strip into the incubation tray, each incubation tray carries one strip. Unused strips should be sealed into the aluminum pouches together with desiccant.
3. Add 1000 µL of Sample Diluent into each incubation tray.
4. Incubate the test strip for 5 minutes at room temperature（20-25℃） with gentle agitation, and then remove it.
5. Add 800 µL of Sample Diluent into each incubation tray, then pipette 200 µL of patient sample and add it into the incubation tray.
6. Incubate for 25 minutes at room temperature （20-25℃）with gentle agitation.
7. Remove diluted samples completely. Wash test strip 3 times using 1500 µL Wash Buffer for 1 minutes with gentle agitation. Remove Wash Buffer after every washing step.
8. Pipette 1000 µL Biotinylated Anti-IgE antibodies.
9. Incubate for 20 minutes at room temperature （20-25℃） with gentle agitation. Remove Biotinylated Anti-IgE antibodies. Repeat washing steps of step 7.
10. Pipette 1000µL Conjugate Solution and incubate for 10 minutes at room temperature with gentle agitation. Remove Conjugate. Repeat washing steps of step 7.
11. Add 1000 µL Substrate Solution and incubate for 15 minutes at room temperature （20-25℃） with gentle agitation.
12. Remove Substrate. Wash with 1000 µL distilled or de-ionized (D.I.) water for 1 minute at room temperature（20-25℃） with gentle agitation. Repeat this wash step for another 2 times.
13. Dry test strip, and stick it onto the Scoring Sheet to record the test results.
14. Judge the total IgE level and specific IgE level of the sample according to the gray value with the interpretation system.

Operation by automatic immunoblotting instrument

Detailed information about operation can be taken from the Instrument User's Manual.

Note that, it is important to perform all routine maintenance procedures for optimal performance.

1. Put the reagents into the corresponding position of the reagent chamber of the fully automatic immunoblotting analyzer and empty the waste liquid bottle.

2. Turn on the power supply of the analyzer and start the operation program of the automatic immunoblotting analyzer.

3. Put the test strip into the corresponding slot of theanalyzer. place the sample to be tested in the sample chamber, edit the sample information, select the item and perform the test.

4. The test results are automatically executed by the system.

5. After the experiment, the sample and reagent shall be recovered and the pipeline shall be flushed.

QUALITY CONTROL PROCEDURES

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

CUT-OFF VALUE DETERMINATION

Clinical significance cut-off of allergen-specific IgE concentration has been set at 0.35 IU/mL for indication for sensitization.

Through the evaluation of 200 clinical samples, the concentration value of clinical samples was analyzed by receiver operating characteristic curve (ROC curve), which confirmed the rationality of the cut off value.

Note: Each laboratory shall determine the usability of cut-off value through experiments. If necessary, it is recommended that each laboratory establish its own cut-off value.

INTERPRETATION OF RESULTS

For each allergen, the coloring intensity on the membrane strip (gray value) is directly proportional to the content of specific IgE antibody in the patient's serum.

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

The positive results of mixed allergens show that the specific IgE antibodies of one or more allergens are positive. When it is necessary to further determine the single allergen specific IgE antibody concentration, it is recommended to use an appropriate single allergen specific IgE antibody detection kit for re-detection. The single detection concentration is not comparable to the mixed item detection concentration, and the mixed item detection concentration cannot be considered as the sum of single concentrations.

Total IgE:

The mouse anti human IgE antibody coated on nitrocellulose membrane binds to the free IgE antibody in the patient's serum. The results are expressed as < 100 IU / ml, 100-200 IU / ml and > 200 IU / ml. The positive result is not less than 100 IU / ml.

PERFORMANCE CHARACTERISTICS

1. ***Appearance and volume:*** The outer package box shall be clean and tidy, label shall be clear and accurate. Each component in the kit shall be complete. Reagent bottle sealed no leakage and the liquid shall be clear and transparent. The strip shall be clean, free of stain and damage.
2. ***Coincidence rate of negative reference materials:*** The coincidence rate of negative reference materials shall be 100%。
3. ***Coincidence rate of positive reference materials:*** The coincidence rate of positive reference materials shall be 100%。
4. ***Detection limit:*** The detection limit shall not be greater than 0.35 IU / ml.
5. ***Correlation:*** Test the reference panel, the difference of a certain sample result should not be more than one class in the same direction. The result of positive samples should not be negative and the result of negative samples should not be positive.
6. ***PRECISION：***

   INTRA-ASSAY: Two repeatability references (weak positive and positive) were taken and tested with 10 replicates for each in a single run. Coefficient of variation (CV) was calculated for each of two samples.CV≤15%.

   INTER-ASSAY: Two repeatability references (weak positive and positive) were taken and tested with 4 replicates in a single run, 2 runs per day for 10 days. Coefficient of variation (CV) was calculated for each of two samples. CV≤15%.
7. ***METHODCOMPARISON:*** Method comparison was implemented by comparing the clinical samples results of the assay to the results of predicated assay. The positive and negative agreement were analyzed, sensitivity and specificity were summarized. Sensitivity ≥ 85%, specificity ≥ 90%.
8. ***Interference:*** No interference has been observed on negative reference and detection limit reference with bilirubin, hemoglobin, triglycerides at the level indicated below: Hemoglobin ≤ 400 mg/L; Bilirubin ≤ 20 mg/dL; Triglycerides ≤ 2,000 mg/dL.

TEST LIMITATIONS

1. 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.

2. 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.
3. When the serum sIgE titer is too high, the antibody will bind to the corresponding allergen band, resulting in the level of total IgE.
4. The total IgE test line result can not replace the ELISA total IgE test method required by the World Health Organization (WHO). It is only used to evaluate the negative / positive predictive value.

SYMBOLS

REF	Catalog Number		Use-by date
IVD	In Vitro diagnostic medical device	LOT	Lot Number
+2°C +8°C	Store between +2°C and +8°C		Consult Instruction for Use
	Manufacturer	EC REP	Authorized Representative in the European Community
CE	CE Marking		Contains Sufficient for<n>Tests
	Biological Risk		GHS07 Warning

Allergen BIOT	Biotinylated allergen

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The eIFU is available on Website:

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TECHNICAL ASSISTANCE

For technical assistance, contact your National Distributor.

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