AccuDiag™ IgE Seasonal Allergens ELISA Kit

REF 5150-8

IVD See External Label 2-8°C 96 Tests

IgE Seasonal Allergens ELISA	
Principle	ImmunoBlot
Detection	Qualitative
Sample	50 µL serum/plasma
Incubation Time	125 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

Diagnostic Automation, Inc. (DAI) IgE Seasonal Allergens kit has been designed for the detection of specific allergen-related IgE antibodies in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of Diagnostic Automation, Inc.

This assay is intended for in-vitro diagnostic use only.

Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

SIGNIFICANCE AND SUMMARY

The existence of IgE in man as a unique class of immunoglobulins which are important in the mediation of the allergic response has been known for over twenty years. The mechanism of action involves an initial antigenic stimulation of immunocompetent B lymphocytes by a specific antigen, a process which induces the lymphocyte to respond by producing specific antibody of several classes.

One class, reaginic or IgE antibody, becomes partially bound via its Fc portion to receptors on the surface of mast cells end basophilic leukocytes. Upon further stimulation by specific allergens, these cell-bound IgE molecules bind via their Fab portion to the allergen. This combination triggers the mast cells and basophilic leukocytes to release various vasoactive amines into the blood and the surrounding tissue. These substances cause smooth muscle constriction and lead ultimately to allergic conditions such as wheal and flare reactions, hives, dermatitis, rhinitis, hay fever, asthma and anaphylactic shock.

IgE determinations are most valuable in the diagnostic assessment of patients with established or suspected allergic disease. In normal subjects, IgE values are related to age, with normal values peaking around 10-14 years. Infants and children with family history of atopic allergy are at increased risk of developing disease and constitute a prime population for screening. Studies have shown that conditions such as asthma, rhinitis, eczema, urticaria, dermatitis and some parasitic infections lead to increased IgE levels. Asthma, hay fever and atopic eczema patients may produce levels 3-10 times those of normal patients.

Circulating levels of total or allergen-specific IgE can be determined by the use of anti-human IgE or specific allergens attached to a solid phase carrier. This approach uses an enzyme labelled antibody towards IgE and is known as the enzyme allegro sorbent test (EAST).

ASSAY PRINCIPLE

The DAI IgE Seasonal Allergens kit is based on the principle of the enzyme immunoassay. 16 patients can be tested with each kit. One test strip is required per patient. On each individual strip 20 different allergens including CCD for the detection of antibodies of low clinical relevance and a control line for test evaluation are coated in parallel lines. Following a pre-wetting step the strips are incubated with patient serum. A binding between the IgE antibodies of the serum and the immobilized allergens takes place. After 60 minutes incubation at room temperature, the strips are rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgE-AP conjugate is added and incubated for another 30 minutes at room temperature. After a further washing step, the substrate (BCIP/NBT) solution is pipetted and incubated for 30 minutes at room temperature, inducing the development of a precipitating dye on the lines in the case of positive reactions. The color development is terminated by rinsing the strips with wash solution. The concentration of the IgE antibodies is directly proportional to the intensity of the color.

SPECIMEN COLLECTION & PREPARATION

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 7 days. For a longer storage they should be kept at -20°C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the fast and sensitive determination the samples are tested diluted 1:5 in sample diluent (100 µL sample + 400 µL sample diluent). If not enough sample volume is available, it can be diluted 1:10 with ready-to-use sample diluent (e.g. 50 µL sample + 450 µL sample diluent) and tested according to the alternative procedure as stated in "Assay Procedure".

REAGENTS

Materials provided with the kit

1. Allergen coated nitrocellulose test strips (16x)
2. Enzyme Conjugate (9 ml)
3. Sample Diluent (60 ml)
4. Washing Buffer (10x) (60 ml)
5. Substrate (18 ml)
6. Incubation Tray (1x)
7. Scanning Template (1x)

Universal Reagents
Washing buffer, sample diluent and substrate are identical for all IgE screen lineblot test kits from Diagnostic Automation, Inc. with Alkaline Phosphatase as detecting enzyme and may be interchanged between products and lots. All other reagents are assigned to a special kit lot and must not be mixed.

1. STRIP **Nitrocellulose Test Strips**
   16 strips for 16 patients. Each individual strip is coated with 20 different allergens including CCD for the detection of antibodies of low clinical relevance and one control antigen (see Distribution Scheme). Ready-to-use
2. CONJ **Enzyme Conjugate**
   9 mL, anti-human-IgE-AP (mouse), in protein-containing buffer solution. Addition of 0.01% methylisothiazolone and 0.01% bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.
3. SAMP DIL **Sample Diluent**
   60 mL, PBS/BSA buffer. Addition of 0.05% sodium azide. Ready-to-use.
4. WASH BUF CONC **Washing Buffer**
   60 mL, TBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with deionized water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes. Diluted washing buffer can be stored at 2-8°C for 4 weeks.
5. SUBS **Substrate**
   18 mL, BCIP/NBT. Ready-to-use.
6. **Incubation Tray**
   With 8 channels for the incubation of 8 test strips.
7. **Scanning Template**
8. **Instruction Manual**

Materials required but not provided

1. Micropipets
2. Tubes for serum dilution (optional)
3. Rocking shaker
4. Vacuum pump
5. Deionized water

REAGENT PREPARATION

Washing Solution: dilute before use 1+9 with deionized water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary

ASSAY PROCEDURE

1. Place the required amount of test strips into the incubation tray. The visible marking on each test strip has to be directed upwards.
2. Fill 1 mL sample diluent into each channel and incubate the test strips on a rocking shaker for 5 minutes at room temperature. Aspirate the solution with the aid of a vacuum pump afterwards.
3. Fill 0.5 mL 1:5 diluted sample into each channel of the tray and incubate the test strips on a rocking shaker for 60 minutes at room temperature. Aspirate the solution with the aid of a vacuum pump afterwards.
   Note: For an easier handling the sample can be diluted and mixed directly in the incubation channel. For this fill 0.4 mL sample diluent into the channel and add 100 µL sample. Start shaking immediately after sample addition.
4. Fill 2 mL diluted washing solution into each channel and rinse the strips for at least 2 minutes on a rocking shaker at room temperature. Aspirate the solution with the aid of a vacuum pump afterwards. Carry out this step three times altogether.
5. Fill 0.5 mL ready-to-use conjugate into each channel and incubate the test strips on a rocking shaker for 30 minutes at room temperature. Aspirate the solution with the aid of a vacuum pump afterwards
6. Repeat the washing step as described in point 4.
7. Fill 1 mL ready-to-use substrate into each channel and incubate the test strips on a rocking shaker for 30 minutes. Aspirate the solution with the aid of a vacuum pump afterwards.
8. Fill 1 mL diluted washing solution into each channel to terminate the substrate reaction. Aspirate the solution with the aid of a vacuum pump.
9. Air dry and evaluate the test strips.

Note: ***The speed of the rocking shaker used in all incubation steps is dependent on the geometry of the individual instrument. Validation experiments showed that a speed of >90/min resulted in reliable results with common devices.***

Alternative procedure: Small sample volumes can be diluted 1:10 in sample diluent and incubated overnight at room temperature (point 3. of the above stated procedure). Conjugate incubation (point 5.) and substrate incubation (point 7.) are prolonged to 60 minutes each. All other steps are performed accordingly.

RESULTS

Moist test strips may show a certain background staining which disappears while drying. For this reason the strips are only evaluated when they are completely dry.

Note that some samples show a background staining, which will not disappear while drying. In this case white bands may occur at the position of the coated allergens. These signals have to be interpreted as negative.

Some samples show very weak reactions to multiple allergens. It cannot totally be excluded that these samples tend to show unspecific reactions and therefore they should to be considered as negative for all allergens with similarly weak reactions. As an aid the CCD line may be considered. A positive signal for the CCD line in combination with multiple other positive reactions is deemed to indicate cross-reacting antibodies with low clinical significance.

The test run has to be considered as valid when a clear visible control line develops during substrate incubation. This line will appear even if all allergens show a negative result. The control line is the nearest signal to the identification code which is directly printed on the test strip.

Visual Method
The control line of the dried test strips is aligned to the marking on the template ("CL"). Lines 1-20 indicate the location of the coated allergens according to the order as stated in the "Distribution Scheme" section. Signals are interpreted for each allergen according to the following scheme:

Class	Signal	Interpretation
0	No signal or weak signals for multiple allergens	Negative
1	Weak signal	Borderline result
2	Moderate signal	Positive result
3	Intense signal	Strong positive result

Scanner Method

The DAI IgE Atopic Allergens test can be evaluated using any open-system blot processors, e.g.: AutoBlot 3000, ProfiBlot™ 48, etc. for quantitative results.

DISTRIBUTION SCHEME

One control line as well as 20 allergens including CCD for the detection of antibodies of low clinical relevance are distributed over the test strip in the following order.

CL	Control
1	Orchard grass (g3)
2	Perenn. Rye grass (g5)
3	Timothy grass (g6)
4	June grass (g8)
5	Cultivated rye (g12)
6	Velvet grass (g13)
7	Corn (g20)
8	Common ragweed (w1)
9	Mugwort sagebrush (w6)
10	English plantain (w9)
11	Carnation
12	Common sunflower
13	Alder (t2)
14	Birch (t3)
15	Hazel (t4)
16	Elm (t8)
17	Willow (t12)
18	Cottonwood (t14)
19	Eucalyptus (t18)
20	CCD (o214)

LIMITATIONS OF THE ASSAY

- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- None of the reagents are based upon human material. Nevertheless samples have to be treated as potentially infectious and precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25°C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the channels of the incubation tray have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and other instrumentation (e.g. scanner for test evaluation).
- The contact of certain reagents, above all the substrate with skin, eye and mucosa has to be avoided.

REFERENCES

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116,
Woodland Hills, California 91367 USA

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