CE IVD 2°C 8°C

Please read instructions for use carefully before starting the assay

ALFA *Basis Set*

Lateral Flow Assay for the qualitative determination of allergen specific IgE in human serum, plasma or whole blood

Single cassette	REF 1800010	Σ 20
Eight-strip cassette	REF 184000	Σ 80

BACKGROUND

The worldwide frequency of allergies increased significantly over the past decades. The term allergy is often used for type I hypersensitivity reactions (immediate type reactions), whose symptoms generally occur within 30-60 min after contact with the allergen. The most frequent symptoms are: hay fever (rhinitis), conjunctivitis, hives (urticaria), allergic asthma and as the most dangerous manifestation anaphylaxis (the anaphylactic shock).

The allergens causing type I hypersensitivity reactions are mostly proteins derived from the natural environment e.g. plant pollen, animal hair, food, mites and insect venoms.

The characteristic of type I allergies is the involvement of allergen specific immunoglobulins (antibodies) of class E (sIgE). Hence, the detection of sIgE is an important tool of modern allergy diagnostics.

INTENDED USE

ALFA (**A**llergy **L**ateral **F**low **A**ssay) is a rapid assay for the qualitative determination of allergen specific immunoglobulin E (sIgE) in human serum, plasma or whole blood. ALFA is a screening test, which enables the user to perform an allergy test very fast and reliable. By choosing appropriate allergen solutions a symptom based diagnosis is possible.

PRINCIPLE

ALFA *Basis Set* is available as single- (REF 1800010; 20 pcs ≙ 20 det.) and eight-strip cassette (REF 184000; 10 pcs ≙ 80 det.) – plus variable allergen solutions.

To perform the test the patient´s sample is transferred to the sample application point of the *Basis Set*. Immediately afterwards, the desired allergen solution is applied. During incubation of 15-20 min the liquid is driven through the device by capillary flow. The allergen specific IgE of the sample binds specifically to its corresponding allergens of the allergen solution. The labelled allergens are retained at the test line (T) by a capture molecule. At the same time, the sIgE bound to the allergen is bound by an antibody coupled to coloured particles (conjugate). The intensity of the colour reaction at the test line is proportional to the amount of immune complexes consisting of ligand tagged allergens, sIgE, and IgE specific conjugate. **The signal intensity ranges from faintly pink (low titre of sIgE) to dark ruby (high titre of sIgE).**

Access conjugate, which is not bound at the test line, forms a dark ruby control line (C) after 20 min of incubation.

KIT COMPONENTS

ALFA *Basis Set*:	Test Unit (ready to use): Single-strip cassette (REF 1800010; 20 pcs ≙ 20 det.) Eight-strip cassette (REF 184000; 10 pcs ≙ 80 det.)
Storage:	At 2-8°C, do not freeze! Use within 1 day after opening the foil pouch!
Shelf life:	To expiry date if stored in foil pouch (unopened).

MATERIAL TO BE ORDERED SEPARATELY

ALFA *Allergens*:	Ready to use solution.
Order number:	18-Code (Please see current list of ALFA allergens.)
Storage:	At 2-8°C, do not freeze!
Shelf life:	To expiry date.

MATERIAL NEEDED BUT NOT PROVIEDED

- Micro pipette and pipette tips for 20 µL
- Capillary or transfer pipette for 25 µL
- Tubes for serum preparation if required
- Equipment for blood withdrawal
- Clock

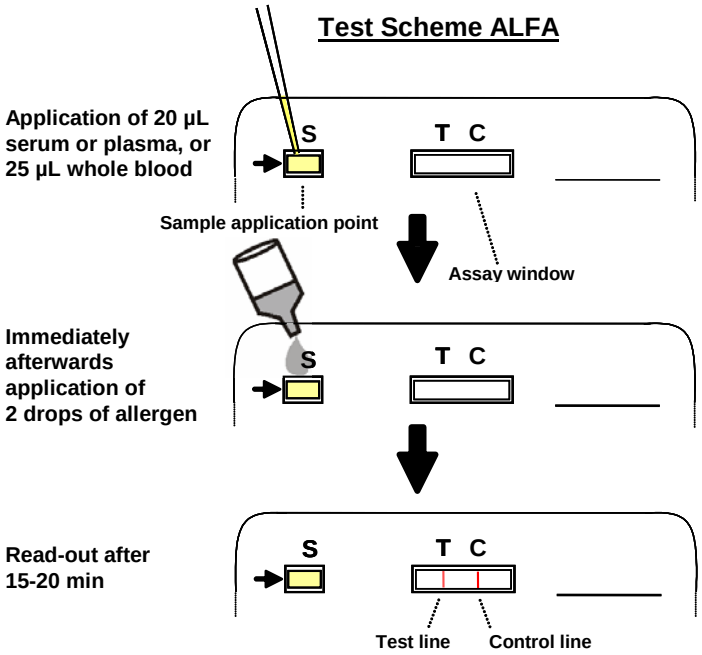
SPECIMEN COLLECTION & PREPARATION

Either serum, plasma or whole blood can be used with this assay. The use of haemolytic or lipemic specimens must be avoided. **Whole blood samples must be collected using a blood capillary or transfer pipette (25 µL) and have to be transferred to the test device immediately. Anticoagulant should not be used**. If serum or plasma is used, proceed as follows:
No additives or preservatives are necessary to maintain the integrity of the specimens if stored at 2-8°C and assayed within 48 hours after collection. If this is not possible or if specimens have to be shipped, samples must be frozen. To perform the assay, thaw and bring samples up to room temperature (RT, 18-25°C). Repeated freezing and thawing should be avoided.

ASSAY PROCEDURE

Attention!
Bring *Basis Set* (in foil pouch) as well as the allergen solution up to RT, at least 30 minutes before starting the assay.

- If the foil pouch is damaged, do not use the test unit.
- Use the test unit within 1 day at RT (18-25°C) after opening the foil pouch.
- Avoid performing the test in direct sunlight.
- Keep Basis Set in a horizontal position and avoid movement of the cassette while running the assay.



1. Transfer **20 µL of serum or plasma, or 25 µL of whole blood** to the sample application point of the *Basis Set*.

2. Immediately afterwards, apply **2 drops** of the desired **allergen solution** to the sample application point of the *Basis Set* (hold dropper bottle 1 cm above the sample application point).

3. The test result becomes visible in the evaluation window after 15-20 min of incubation.

The test result has to be interpreted within 15 to 20 min after application of the allergen solution. Any assessment done later or earlier can cause erroneous interpretation of the result!

INTERPRETATION OF THE RESULTS

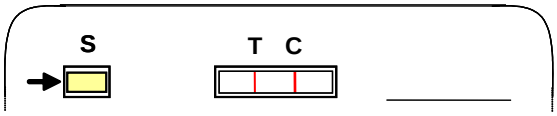
Attention! The test result is only valid, if the control line (C) of the test unit is clearly visible!

The results of ALFA have to be evaluated on the basis of the signal intensity of the test line (T). The intensity of the colour correlates with the amount of sIgE in the sample. Attention should be drawn to:

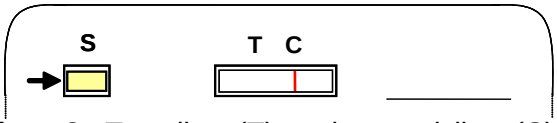
- Weak signals may become visible not before 20 min of incubation.
- In most cases, the colour of the control line is more intensive than of the test line
- Strong positive results may become visible before 20 min of incubation.
- Hold test device towards the light

Possible test results can be:

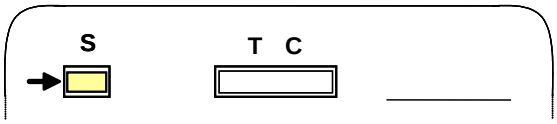
Variant 1: Test line (T) and control line (C) are coloured.
➔ Test result is **positive**; the patient sample contains sIgE against the allergen solution used:



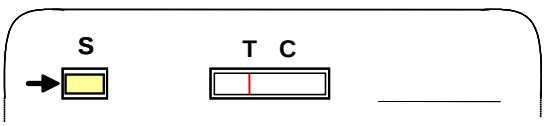
Variant 2: Test line (T) is not coloured but control line (C) is coloured.
➔ Test result is **negative**; the patient sample does not contain measurable amounts of sIgE against the allergen solution used:



Variant 3: Test line (T) and control line (C) are not coloured.
➔ The test result is **invalid**; the test has to be repeated with a new *Basis Set*:



Variant 4: Test line (T) is coloured but control line (C) is not coloured.
➔ The test result is **invalid**; the test has to be repeated with a new *Basis Set*:



With the aid of the LFA Reader (REF 190001 or 190002) the test result can be interpreted semi-quantitatively in U/mL. The measurement has to be performed according to the instruction for use of the LFA Reader.

ASSAY PERFORMANCE

Table 1 summarizes performance data (analytical sensitivity and specificity) for the most important allergens as well as two allergen screens using serum as specimen. For more details see relevant product information or related publications.

Table 1 Analytical sensitivity and specificity of ALFA

ALFA Single Allergens/ Allergen Screens	Sensitivity (%) compared to ImmunoCAP® and ALLERG-O-LIQ	Specifictiy (%) compared to ImmunoCAP® and ALLERG-O-LIQ
D. pteronyssinus (d1)	93.3	97.2
Cat (e1)	95.0	100.0
Timothy grass (g6)	96.7	100.0
Birch (t3)	93.6	98.0
Alternaria alternata (m6)	88.9	100
Perennial Screen	88.4	97.2
Seasonal Screen	94.3	98.8

Detection limit of ALFA is ≈ RAST class 2. Test results have to be verified on the basis of the patient's history. Possibly, other *in-vitro* diagnostics should be taken into consideration.
The performance can vary between serum, plasma and whole blood samples.

LIMITATIONS OF THE METHODE

High titre of allergen specific IgG can mask sIgE. Total IgE titre above 8000 IU/mL can lead to false negative results. Negative test results can not exclude a weak sensitization of the patient against the tested allergen.

LITERATURE

1. Hamilton RG, Franklin Adkinson N Jr. **In-vitro assays for the diagnosis of IgE-mediated disorders**. *J Allergy Clin Immunol* 2004; **114**:213-225.
2. Lucassen R, Fooke M, Kleine-Tebbe J, Mahler M. **Development and Evaluation of a Rapid Assay for the Diagnosis of IgE-mediated Type I Allergies**. *J Investig Allergol Clin Immunol* 2008; **18**:223-230.
3. Lucassen R, Fooke M, Lorenz C, Kleine-Tebbe J and Mahler M. **Evaluation of a rapid assay for the diagnosis of type I allergy**. Abstract: EAACI 2008 Barcelona, Spain
4. Pfender N, Lucassen R, Offermann N, Schulte-Pelkum J, Fooke M, Jakob T: **Evaluation of a novel rapid test system for the detection of specific IgE to hymenoptera venoms.** Journal of Allergy 2012, 2012: 1-7.

PRECAUTIONS FOR USERS

1. In compliance with article 1 paragraph 2b European directive 98/79/EC the use of *in-vitro* diagnostic medical devices is intended to secure suitability, performance and safety of the product by the manufacturer. Therefore the test procedure, information, precautions and warnings stated in the instructions for use have to be followed strictly. The kit has only to be used as described on page 1 (intended use).
2. The test must be performed according to this instruction, which contains all necessary information, precautions and warnings. The use of the test kit with analyzers and similar equipment has to be validated. Any change in design, composition of the test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes resulting in false results and other incidents.
3. The kit is intended for use by trained and qualified professionals carrying out research or diagnostic activities only. Pregnant women should not perform the test.
4. Laboratory equipment has to be maintained according to the manufacturer's instructions and must be tested for its correct function before use.
5. For *in-vitro* diagnostic use only. Use only once. Do not use components exceeding the expiry date. Do not combine reagents of other suppliers or kit components of different lots (unless specified on page 1) with this kit.
6. Do not use kit components when the package of the component is damaged. Cap vials tightly immediately after use to avoid evaporation and microbiological contamination. Do not interchange screw caps of the reagent vials.
7. Test components based on human serum were tested using a CE marked method for the presence of antibodies against HIV 1 / HIV 2, Anti-HBc, and Anti-HCV as well as for hepatitis antigen HBsAg and were found to be negative. Nevertheless, material based on human serum should be handled as potentially infectious (BIOHAZARD).
8. Some kit components may contain bovine serum albumin, of which according to the manufacturer no infectious potential is known. Due to the eventual occurrence of undetectable infectious agents we recommend to handle any product of animal origin as potentially infectious.
9. The following safety rules should be followed with all reagents:
   - Do not get in eyes, on skin, or on clothing (P262). Do not breathe spray (P260).
   - IF SWALLOWED: rinse mouth. Do NOT induce vomiting (P301/330/331)
   - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower (P303/361/353).
   - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing (P303/340).
   - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305/351/338)
   - Don't eat, drink or smoke while performing the test. Keep away from food, feed and beverage.
   - Wear protective gloves/protective clothing/eye protection (P280). Wash hands thoroughly after handling (P264) and care for your skin.
10. The preservatives (Bronidox L, Azid) are toxic to aquatic life, but their concentration is not hazardous to environment anymore. On disposal, flush large volumes of reagents with plenty of water.
11. Waste containing serum must be collected in separate containers containing an appropriate disinfectant in sufficient concentration. This material has to be treated according to national biohazard and safety guidelines or regulations.
12. We refer to the national regulations of medical devices regarding *in-vitro* diagnostic test kits.

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Lot- Number	European conformity	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	Sufficient for <n> tests	Manu-factured by	Bio-hazard