ALEX

ALLERGY EXPLORER

INSTRUCTION FOR USE

CE IVD

Powered by MADX MACRO ARRAY DIAGNOSTICS

INTENDED USE

The Allergy Explorer (ALEX) is a quantitative in vitro diagnostic test for the measurement of allergen specific IgE (sIgE) and a semi-quantitative in vitro diagnostic test for the measurement of total IgE (tIgE) in human serum or plasma (exception EDTA-plasma). It is to be used by clinical chemistry laboratories, trained laboratory personnel and medical professionals for the purpose of supporting the clinical diagnosis of IgE mediated diseases, in conjunction with other clinical findings or diagnostic test results.

All major allergic sensitizers are covered by the ALEX allergen panel. Please contact **support@macroarraydx.com** to obtain the complete list of extracts and molecular allergens present on ALEX.

SUMMARY AND EXPLANATION OF THE TEST

Allergic reactions are immediate type I hypersensitivity reactions and are mediated by antibodies belonging to the IgE class of immunoglobulins. After exposure to specific allergens, IgE–mediated release of histamine and other mediators from mast cells and basophils results in clinical manifestations such as asthma, allergic rhinoconjunctivitis, atopic eczema and gastro intestinal symptoms [1]. Therefore, a detailed sensitization pattern to specific allergens assists in the evaluation of allergic patients [2-6].

Important information for the user

For the correct use of ALEX, it is necessary for the user to carefully read and follow this instructions for use. The manufacturer assumes no liability for any use of this test system which is not described in this document or for modifications by the user of the test system.

PRINCIPLE OF THE PROCEDURE

ALEX is a solid-phase immunoassay. Allergens extracts or molecular allergens, which are coupled to nano-particles, are deposited in a systematic fashion onto a solid phase forming a macroscopic array. First, the particle bound allergens react with specific IgE that is present in the patient's sample. After incubation, non-specific IgE is washed off. The procedure continues by adding an enzyme labelled anti-human IgE detection antibody which forms a complex with the particle bound specific IgE. After a second washing step, substrate is added which is converted to an insoluble, coloured precipitate by the antibody-bound enzyme. Finally, the enzyme-substrate reaction is stopped by adding a blocking reagent. The amount of precipitate is proportional to the concentration of specific IgE in the patient sample. The lab test procedure is followed by image acquisition and analysis using the ImageXplorer device. The test results are analyzed with MADx's Raptor Software and reported in IgE response units (kU_A/L). Total IgE results are also reported in IgE response units (kU/L).

SHIPMENT AND STORAGE

The shipment of ALEX takes place at ambient temperature conditions. Nevertheless, the kit has to be stored immediately upon delivery at 2-8°C.

Stored correctly, ALEX and its components can be used until the indicated expiration date.

Kit reagents are stable for 6 months after opening (at the indicated storage conditions).

Except of ALEX Cartridges, as they are single packed.

WASTE DISPOSAL

Dispose the used ALEX cartridge and unused kit components with laboratory chemical waste.

Follow all national, state, and local regulations regarding disposal.

GLOSSARY OF SYMBOLS

The meaning of the symbols stays the same, regardless of colour

Symbol | Description

CE mark

In vitro diagnostic medical device

Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.

Contains sufficient for <n> tests

Indicates the total number of IVD tests that can be performed with the IVD.

Consult instructions for use

Indicates the need for the user to consult the instructions for use.

Do not use if package is damaged

Indicates a medical device that should not be used if the package has been damaged or opened.

Catalogue number

Indicates the manufacturer's catalogue number so that the medical device can be identified.

Temperature limit

Indicates the temperature limits to which the test (reagents) can be safely exposed to.

Use-by date

Indicates the date after which the test (reagents) is not to be used.

Batch code

Indicates the manufacturer's batch code so that the batch or lot can be identified.

Do not re-use

Indicates a test that is intended for one use, or for use on a single patient during a single procedure.

Warning (GHS pictogram)

Refer to safety data sheet for details

Manufacturer

Indicates the medical device manufacturer, as defined in EU Directives 90/385/ EEC, 93/42/EEC and 98/79/EC.

Important Note

For IVD performance evaluation only

Indicates an IVD that is intended to be used only for evaluating its performance characteristics before it is placed on the market for medical diagnostic use.

ALEX Kit components

Each component (reagent) is stable until the date stated on each individual component's label. It is not recommended to pool any reagents from different kit lots. For a list of allergen extracts and molecular allergens immobilized on the ALEX array, please contact **support@macroarraydx.com**.

ALEX Test Kit	For 20 analyses, calibration integrated on every array; packed and sealed.	Ready for use. Store at 2-8°C until expiry date.
ALEX Washing Solution	2 bottles á 50 mL	Ready for use. Store at 2-8°C until expiry date. Allow washing solution to reach room temperature before use. Opened reagent is stable for 6 months at 2-8°C.
ALEX Sample Diluent	1 bottle á 9 mL	Ready for use. Store at 2-8°C until expiry date. Allow sample diluent to reach room temperature before use. Opened reagent is stable for 6 months at 2-8°C, includes CCD inhibitor.
ALEX Detection Antibody	1 bottle á 11 mL	Ready for use. Store at 2-8°C until expiry date. Allow detection antibody to reach room temperature before use. Opened reagent is stable for 6 months at 2-8°C.
ALEX Substrate Solution	1 bottle á 11 mL	Ready for use. Store at 2-8°C until expiry date. Allow substrate solution to reach room temperature before use. Opened reagent is stable for 6 months at 2-8°C.
ALEX Stop Solution	2 vials á 1.2 mL	Ready for use. Store at 2-8°C until expiry date. Allow stop solution to reach room temperature before use. Opened reagent is stable for 6 months at 2-8°C. May appear as a turbid solution after prolonged storage. This has no effect on results.

Required equipment for processing and analysing ALEX

- Arrayholder (optional)
- ImageXplorer
- Lab Rocker (inclination angle 8°, required speed 8 rpm)
- Incubation chamber (WxDxH – 35x25x2 cm)
- Raptor analysis software
- PC/Laptop

ALEX Starter kit contains all required equipment, except PC/Laptop – **REF 01-0010-00**.

Single items of the Starter kit are available on request. PC/Laptop is provided only on request.

Required equipment, not provided by MADx

- Purified Water
- Pipettes & tips (100 µL & 1000 µL)

Maintenance services according to manufacturer's instructions.

Handling of ALEX arrays

Do not touch the array surface. Any surface defects caused by blunt or sharp objects can interfere with the correct readout of the results.

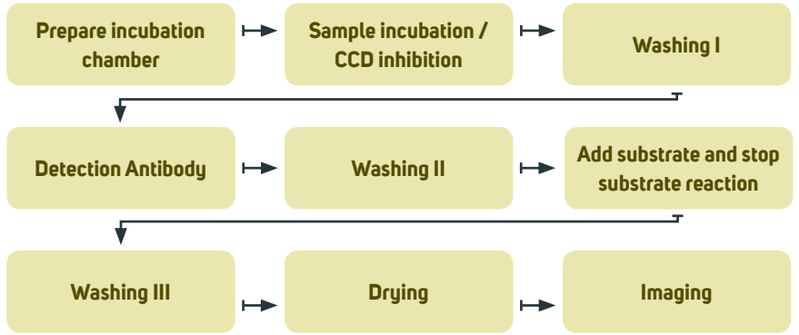
Do not acquire ALEX images before array is completely dry (dry at room temperature).

Warnings and precautions

- It is recommended to wear hand and eye protection as well as lab coats and follow good laboratory practices when preparing and handling reagents and samples.
- In accordance with good laboratory practice, all human source material should be considered potentially infectious and handled with the same precautions as Patient samples.
- The sample diluent is partially prepared from human blood sources. The product was tested non-reactive for Hepatitis B Surface Antigen (HBsAg), antibodies to Hepatitis C (HCV) and antibodies to HIV-1/HIV-2.
- Sample diluent and washing solution contain sodium azide as a preservative and must be handled with care. Safety data sheet is available upon request.
- For in vitro diagnostic use only. Not for internal or external use in humans or animals.
- Only personnel trained in laboratory practice should use this kit.
- Upon arrival, check the kit components for damage. If one of the components is damaged (e.g. buffer bottles), contact MADx (**support@macroarraydx.com**). Do not use damaged kit components, as their use may lead to poor kit performance.
- Do not use reagents beyond their expiry dates.
- Do not mix reagents from different batches.

ASSAY PROCEDURE

Flowchart of Assay Procedure



Preparation:

Preparation of samples: Serum or plasma (heparin, citrate, no EDTA) samples from capillary or venous blood can be used. Blood samples can be collected using standard procedures. Store samples at 2–8°C for up to one week. Keep serum and plasma samples at -20°C for prolonged storage. Shipment of serum/plasma samples at room temperature is applicable. Always allow samples to reach room temperature before use.

Incubation chamber: Close lid for all assay steps to prevent drop in humidity.

Parameters of Procedure

- 100 µL sample + 400 µL ALEX Sample Diluent
- 500 µL ALEX Detection Antibody,
- 500 µL ALEX Substrate Solution,
- 100 µL ALEX Stop Solution
- 4500 µL ALEX Washing Solution

Assay time is approximately 3 h 30 min.

It is not recommended to run more assays than can be pipetted in 10 min.

All incubations are performed at room temperature, 20-26°C.

ALEX ASSAY PROCEDURE

All reagents are to be used at room temperature (20-26°C). The assay must not be performed in direct sunlight.

1. **Prepare incubation chamber**

   Open incubation chamber and place paper towels on bottom part. Soak paper towels with purified water until no dry parts of the paper towels are visible.

2. **Sample incubation/CCD inhibition**

   Take out the needed number of ALEX tests and place them into the array holder(s). Add 400 µL of ALEX sample diluent to each cartridge. Add 100 µL patient sample to the cartridges. Ensure that the resulting solution is spread evenly. Place the cartridges onto the lab rocker and start the serum incubation with 8 rpm for 2 hours. Close incubation chamber before starting the lab rocker. After 2 hours, discharge the patient samples into a collection container. Carefully wipe off droplets from the cartridge using a paper towel.

Avoid touching the array surface with the paper towel!

Avoid any carry over or cross-contamination of patient samples between individual ALEX cartridges!

Optional: with the standard CCD antibody inhibition protocol (as described in paragraph 2: sample incubation/ CCD inhibition) the CCD inhibition efficiency is 85%. If a higher rate of inhibition efficiency is required, prepare a 1 mL sample tube, add 400 µL sample diluent and 100 µL serum. Incubate for 30 minutes (non-shaking) and then proceed with the usual assay procedure.

Note: The extra CCD inhibition step leads to an inhibition rate for CCD antibodies of above 95%.

3. **Washing I**

   Add 500 µL ALEX Washing Solution to each cartridge and incubate on the lab rocker (at 8 rpm) for 5 minutes. Discharge the washing solution into a collection container and vigorously tap the cartridges on a stack of dry paper towels.

   Repeat this step 2 more times.

4. **Add detection antibody**

   Add 500 µL of ALEX Detection Antibody to each cartridge.

Make sure that the complete array surface is covered by the ALEX Detection Antibody solution.

Place the cartridges into the incubation chamber on the lab rocker and incubate them at 8 rpm for 30 minutes. Discharge the detection antibody solution into a collection container. Carefully wipe off remaining droplets from the cartridges using a paper towel.

5. Washing II

Add 500 µL ALEX Washing Solution to each cartridge and incubate on the lab rocker at 8 rpm for 5 minutes. Discharge the washing solution into a collection container and vigorously tap the cartridges on a stack of dry paper towels.

Repeat this step 4 more times.

6. Add substrate and stop substrate reaction

Add 500 µL of ALEX Substrate Solution to each cartridge.

Start a timer after filling the first cartridge and proceed with the filling of the remaining cartridges. Make sure that the complete array surface is covered by the substrate solution and incubate the arrays for 8 minutes on the lab rocker (8 rpm).

After **exactly 8 minutes**, add 100 µL of the ALEX Stop Solution to all cartridges, starting with the first cartridge to assure that all arrays are incubated for the same time with the substrate solution. Carefully agitate to evenly distribute the stop solution in the array cartridges, after the stop solution was pipetted onto all arrays.

Afterwards discharge substrate/stop solution from the cartridges and wipe off any remaining droplets from the cartridges using a paper towel.

7. Washing III

Add 500 µL ALEX Washing Solution to each cartridge and incubate on the lab rocker at 8 rpm for 30 seconds.

Discharge the washing solution into a collection container and vigorously tap the cartridges on a stack of dry paper towels.

8. Image analysis

After finishing the assay procedure, air dry the arrays at room temperature until they are completely dry (can take up to 45 min).

This is essential for the sensitivity of the procedure as only completely dry arrays provide a superior signal-to-noise ratio.

Finally, the completely dried arrays are scanned with the ImageXplorer and analysed with the Raptor software. For details please see the Raptor software manual.

The Raptor software is verified with the MADx ImageXplorer only, and consequently Macro Array Diagnostics will take no responsibility for the results, if another imaging system is used (e.g a flatbed scanner).

Assay Calibration

Systematic variations in signal levels between lots are normalized by heterologous calibration against an IgE reference curve. A curve fit is calculated and the resulting equation applied to transform arbitrary intensity units into quantitative units. Curve parameters for each lot are adjusted by in-house reference testing against a serum preparation tested on ImmunoCAP for specific IgE against several allergens. The ALEX results are therefore indirectly traceable against the WHO reference preparation 75/502 for total IgE.

Lot specific calibration parameters are encoded in the barcode and referenced against an IgE standard curve present on each test cartridge to adjust for assay variations. ALEX sIgE test results are expressed as kU_A/L. Total IgE results are semi-quantitative and calculated from two different anti-IgE measurements with lot-specific calibration factors encoded in the ALEX barcode.

Measuring Range

Specific IgE: 0.3-50 kU_A/L, quantitative

Total IgE: 1-2500 kU/L, semi-quantitative

QUALITY CONTROL

Record keeping for each assay

According to good laboratory practice it is recommended to record the lot numbers of all reagents used.

Control Specimens

According to good laboratory practice it is recommended that quality control samples are included within defined intervals.

DATA ANALYSIS

For the image analysis of processed arrays, the ImageXplorer is to be used. ALEX images are automatically analysed using MADx's Raptor software and a report is generated summarising the results for the user.

RESULTS

ALEX is a quantitative method for specific IgE and semi-quantitative method for total IgE. Allergen specific IgE antibodies are expressed as IgE response units (kU_A/L), total IgE results as kU/L. MADx's Raptor software automatically calculates and reports sIgE results (quantitatively) and tIgE results (semi- quantitatively).

LIMITATIONS OF THE PROCEDURE

A definitive clinical diagnosis should only be made in conjunction with all available clinical findings by medical professionals and shall not be based on results of a single diagnostic method only.

In certain areas of application (e.g. food allergy), circulating IgE antibodies may remain undetectable although a clinical manifestation of food allergy against a certain allergen may be present, because these antibodies may be specific to allergens that are modified during industrial processing, cooking or digestion and hence do not exist in the original food for which the patient is tested.

Negative venom results only indicate undetectable levels of venom specific IgE antibodies (e.g. due to long term non-exposure) and do not preclude the existence of clinical hypersensitivity to insect stings.

EXPECTED VALUES

The close association between allergen specific IgE antibody levels and allergic disease is well known and is described thoroughly in literature [1].

Each sensitized patient will show an individual IgE profile when tested with ALEX.

The IgE response with samples from healthy non-allergic individuals will be below 0.3 kU_A/L for single molecular allergens and for allergen extracts when tested with ALEX.

Good laboratory practice recommends that each laboratory establishes its own range of expected values.

PERFORMANCE CHARACTERISTICS

Precision

The below listed mean coefficients of variation (CV) were determined for representative serum samples (n=96) using same lots of reagents. Samples from multi-sensitized patients were tested in triplicates in 10 different assay runs [7].

Concentration - kU_A/L	Intra CV%	Inter CV%	Total CV%
0.3 – 1.0	20	11	25
1 -10	10	6.3	16
>10	6.9	3.9	11

Analytical Sensitivity

The Limit of Detection was determined in accordance with CLSI guideline EP17-A [8] for representative allergen components and was below 0.3 kU_A/L for all allergen components and all allergen extracts.

Analytical Specificity

There is no detectable cross-reactivity with other human Immunoglobulins (IgA, IgG1, IgG2, IgG3, IgG4 and IgM) at normal physiological concentrations.

Interference

There is no detectable interference with bilirubin, cholesterol/triglycerides and haemoglobin at normal physiological concentrations.

Neither is there an interference with tIgE which was tested in concentrations of up to 3000 kU/L.

WARRANTY

The herein presented performance data were obtained using the procedure outlined in this Instructions for Use. Any change or modification in the procedure may affect the results and Macro Array Diagnostics disclaims all warranties expressed (including the implied warranty of merchantability and fitness for use) in such an event. Consequently, Macro Array Diagnostics and its authorized distributors shall not be liable for damages indirect or consequential in such an event.

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ALEX

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MADx

MACRO ARRAY DIAGNOSTICS

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To avoid substantially lengthening this instructions for use, the complete list of extracts and allergens present on ALEX is not stated in this document.

Please contact **support@macroarraydx.com** to obtain the complete list of extracts and allergens present on ALEX.