



ALEX³ ALLERGY XPLOER

INSTRUCTION FOR USE

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I. LANGUAGE DISCLAIMER

This Instruction for Use (IFU) is provided in multiple languages according to regulation (EU) 2017/746. In the event of any discrepancies or inconsistencies between the English version and any translated version, the English version shall prevail and be considered the authoritative reference.

II. LIABILITY STATEMENT

This IFU has been reviewed for accuracy. The instructions for the ALEX³ Allergy Xplorer were correct at the time of publication. Subsequent versions of this guide may be updated without prior notice.

The ALEX³ Allergy Xplorer kit is an in-vitro diagnostic device intended for use by trained laboratory personnel only. The ALEX³ Allergy Xplorer kit may only be used for its intended purpose in accordance with this IFU. The IFU must be observed without exception. If you are unfamiliar with the use of the ALEX³ Allergy Xplorer kit, you are obliged to obtain information from MacroArray Diagnostics (MADx) before using it. MADx assumes no liability for improper use of the ALEX³ Allergy Xplorer kit. MADx shall only be liable for any harm or damage to property directly or indirectly resulting from errors in this IFU in the event of gross negligence or intent, and for personal injury only within the scope of the mandatory statutory provisions.

If any term or provision in this IFU shall be held to be illegal or unenforceable, in whole or in part, under any enactment or rule of law, such term or provision or part shall to that extent be deemed not to form part of this IFU but the enforceability of the remainder of this IFU shall not be affected.

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III. DESCRIPTION

The ALEX³ Allergy Xplorer (ALEX³) is an Enzyme-Linked Immunosorbent Assay (ELISA) – based in-vitro diagnostic test for the quantitative measurement of allergen-specific IgE (sIgE). It can be used as a manual assay in combination with the ImageXplorer device (REF 11-0000-01), or as an automated assay with the MAX 9k (REF 17-0000-01) or MAX 45k (REF 16-0000-01) systems.

This Instruction for Use is applicable for the following products:

Basic UDI-DI	REF	Product
91201229203JS	03-2001-01	ALEX ³ for 20 Analyses
	03-5001-01	ALEX ³ for 50 Analyses



IV. INTENDED PURPOSE

ALEX³ Allergy Xplorer is a test kit used for in-vitro examination of human serum or plasma (exception EDTA-plasma) to provide information to aid the diagnosis of patients suffering from IgE-mediated diseases in conjunction with other clinical findings or diagnostic test results.

The IVD medical device detects allergen-specific IgE (sIgE) quantitatively and total IgE (tIgE) quantitatively in the range from 2 - 1000 kU/l (semi-quantitative 1001-2500 kU/l). The product is used by trained laboratory personnel and medical professionals in a medical laboratory.

V. 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 manifestation such as asthma, allergic rhino-conjunctivitis, atopic eczema, and gastrointestinal symptoms [1]. Therefore, a detailed sensitization pattern to specific allergens assists in the evaluation of allergic patients [2-6]. There is no restriction on the test population. When developing IgE assays, age and sex are typically not considered as critical factors because IgE levels, which are measured in these assays, do not significantly vary based on these demographics.

All major type I allergen sources are covered by ALEX³. A complete list of ALEX³ allergen extracts and molecular allergens can be found at the bottom of this instruction.

Important information for the user!

For the correct use of ALEX³, it is necessary for the user to carefully read and follow these 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.

Attention: The kit variant 03-2001-01 of the ALEX³ test (20 Arrays) is exclusively intended for manual processing. To use this ALEX³ kit variant with the automated MAX 9k, the Washing Solution (REF 00-5003-01) and the Stop Solution (REF 00-5007-01) need to be ordered separately. All further product information can be found in the corresponding instructions for use: <https://www.madx.com/extras>.

The ALEX³ kit variant 03-5001-01 (50 arrays) is to be used for automated processing with MAX 9k (REF 17-0000-01) as well as MAX 45k (REF 16-0000-01) only, under no circumstance with the ImageXplorer device (REF 11-0000-01).

VI. PRINCIPLE OF THE PROCEDURE

ALEX³ is an immunoassay test based on Enzyme-Linked Immunosorbent Assay (ELISA). Allergen extracts or molecular allergens, which are coupled to nanoparticles, 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, colored 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's sample. The lab test procedure is followed by image acquisition and analysis using either the manual system (ImageXplorer) or the automated system (MAX 45k or MAX 9k). The test results are analyzed with RAPTOR SERVER Analysis Software and reported in IgE response units (kU_A/l). Total IgE results are also reported in IgE response units (kU/l). RAPTOR SERVER is available in version 1, for the full four-digit version number please refer to the RAPTOR SERVER imprint available at www.raptor-server.com/imprint.

VII. SHIPMENT AND STORAGE

The shipment of ALEX³ takes place at ambient temperature conditions. Nevertheless, the kit must be stored immediately upon delivery at 2-8°C. Stored correctly, ALEX³ 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).
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Shipment and Storage of Samples

Sample type	Serum	Plasma (Citrate)	Plasma (Heparin)
Short-term storage	1 week at 2-8°C (prevent microbial growth)		
Long-term storage	25-37 years at -20°C [7-8]		
Transport	17 days at room temperature [9]		

VIII. WASTE DISPOSAL

To ensure safe disposal of the device, users must adhere to relevant local, regional, and national regulations concerning waste management and environmental protection. Special attention must be given to any components or consumables that may present a risk of infection or microbial contamination (e.g., consumables contaminated with human serum samples). Used ALEX³ cartridges and any unused kit components should be disposed of as laboratory chemical waste.



IX. GLOSSARY OF SYMBOLS

	Warning (GHS pictogram) Consult the Safety Data Sheet for more information.
	Catalogue number
	Sufficient for <n> tests
	Do not use if packaging is damaged
	In-vitro diagnostic medical device
	CE mark (Notified Body 2962: QMD Services GmbH, Zelinkagasse 10/3, 1010 Vienna, Austria)
	Batch code
	Consult instructions for use
	Manufacturer
	Date of manufacture
	Do not re-use
	Cartridge



	Use-by date
	Temperature limit
	Caution
	Unique device identifier
	ALEX ³ Icon
	MacroArray Diagnostics (MADx)

X. KIT COMPONENTS

New Nomenclature for Reagent Lots

Attention: We are introducing a new lot nomenclature for all MADx reagents (the nomenclature for the cartridges is not affected).

ALEX³ kits with lot number 03EAA01 and subsequently produced lots will be affected by this change.

Key Details:

- **No change for cartridge labels**
- The specific reagents of one reagent lot will show the same label nomenclature and can be combined with different cartridge lots.
We will only vary **position 1 and 2** of our **three-letter code** for the reagents. For instance:



- Reagents with labels **DAA** can be combined with the cartridge lots **DAA, DAB, DAC, DAD, ... up to DAT**.
- Reagents with labels **DBA** can be combined with the cartridge lots **DBA, DBB, DBC, DBD, ... up to DBT**.
- The RAPTOR SERVER Analysis Software has already been updated to reflect these changes. **No action is required from customers.**
The RAPTOR SERVER will recognize and combine the correct cartridges with the corresponding reagents.

Each component (reagent) is stable until the date stated on each individual component's label. Do not combine or mix reagents from different reagent lots (different first two letters). For a comprehensive list of allergen extracts and molecular allergens immobilized on the ALEX³ array, please contact pm@macroarraydx.com.

Kit Components REF 03-2001-01	Content	Properties (reactive ingredients are underlined)
ALEX ³ Cartridge	2 Blisters à 10 ALEX ³ for 20 analyses in total. Calibration via master curve available via RAPTOR SERVER Analysis Software.	Polystyrol cartridge containing a <u>solid-phase immunoassay</u> . Ready for use. Store at 2-8°C until expiry date.
ALEX ³ Sample Diluent	1 bottle à 9 ml	<u>CCD Blocker</u> , TBS-Tween (0.2%) Buffer, < 0.1 % Sodium Azide. Ready for use. Store at 2-8°C until expiry date. Allow reagent to reach room temperature before use. Opened reagent is stable for 6 months at 2-8°C.
Washing Solution	2 bottle à 50 ml	<u>TBS-Tween (0.2%) Buffer</u> , < 0.1 % Sodium Azide. Ready for use. Store at 2-8°C until expiry date. Allow reagent to reach room temperature before use. Opened reagent is stable for 6 months at 2-8°C.
ALEX ³ Detection Antibody	1 bottle à 11 ml	<u>Human Anti-IgE detection antibody</u> in Conjugate Buffer with Additives. Ready for use. Store at 2-8°C until expiry date. Allow reagent to reach room temperature before use. Opened reagent is stable for 6 months at 2-8°C.
ALEX ³ Substrate Solution	1 bottle à 11 ml	<u>NBT/BCIP Substrate</u> (NBT: 4-Nitro blue tetrazolium chloride, solution, BCIP: 5-



Kit Components REF 03-2001-01	Content	Properties (reactive ingredients are underlined)
		bromo-4-chloro-3-indolyl-phosphate, 4-toluidine salt). Ready for use. Store at 2-8°C until expiry date. Allow reagent to reach room temperature before use. Opened reagent is stable for 6 months at 2-8°C.
(ALEX ³) Stop Solution	1 bottle à 2.4 ml	<u>Ethylenediaminetetraacetic acid (EDTA)</u> -Solution Ready for use. Store at 2-8°C until expiry date. Allow reagent 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.

Kit Components REF 03-5001-01	Content	Properties (reactive ingredients are underlined)
ALEX ³ Cartridge	5 Blisters à 10 ALEX ³ for 50 analyses in total. Calibration via master curve available via RAPTOR SERVER Analysis Software.	Polystyrol cartridge containing a <u>solid-phase immunoassay</u> . Ready for use. Store at 2-8°C until expiry date.
ALEX ³ Sample Diluent	1 bottle à 30 ml	<u>CCD Blocker</u> , TBS-Tween (0.2%) Buffer, < 0.1 % Sodium Azide. Ready for use. Store at 2-8°C until expiry date. Allow reagent to reach room temperature before use. Opened reagent is stable for 6 months at 2-8°C.
Washing Solution	4 x conc. 1 bottle à 250 ml	<u>TBS-Tween (0.2%) Buffer</u> , < 0.1 % Sodium Azide. Store at 2-8°C until expiry date. Dilute 1 to 4 with demineralized water before use (250ml Washing Solution 4x conc. + 750ml demineralized water). Allow reagent to reach room temperature before use. Opened reagent is stable for 6 months at 2-8°C.



Kit Components REF 03-5001-01	Content	Properties (reactive ingredients are underlined)
ALEX ³ Detection Antibody	1 bottle à 30 ml	<u>Human Anti-IgE detection antibody</u> in Conjugate Buffer with Additives. Ready for use. Store at 2-8°C until expiry date. Allow reagent to reach room temperature before use. Opened reagent is stable for 6 months at 2-8°C.
ALEX ³ Substrate Solution	1 bottle à 30 ml	<u>NBT/BCIP Substrate</u> . Ready for use. Store at 2-8°C until expiry date. Allow reagent to reach room temperature before use. Opened reagent is stable for 6 months at 2-8°C.
(ALEX ³) Stop Solution	1 bottle à 10 ml	<u>EDTA-Solution</u> . Ready for use. Store at 2-8°C until expiry date. Allow reagent 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.



XI. REQUIRED EQUIPMENT FOR PROCESSING AND ANALYSING

Accessories	Description
Accessories/Products for <u>MANUAL</u> processing	
ImageXplorer (REF 11-0000-01)	Imaging device specifically built to acquire images of ALEX technology-based test cartridges for the <u>manual</u> handling
Array holder (optional)	Product for easier handling of the cartridges during <u>manual</u> processing
Lab Rocker	Vertical rocker is necessary during <u>manual</u> test procedure (WxDxH – 28x15x33 cm, inclination angle 8°, required speed 8 rpm).
Incubation chamber	Necessary during <u>manual</u> test procedure (WxDxH – 35x25x2 cm)
Accessories/Products for <u>AUTOMATED</u> processing	
MAX devices: MAX 45k (REF 16-0000-01) MAX 9k (REF 17-0000-01)	Instruments for the <u>automated</u> processing of ALEX technology-based test cartridges
Washing Solution 4x conc. (REF 00-5003-01)	Reagents for the <u>automated</u> processing of ALEX technology-based test cartridges. The Washing and Stop Solution are components of the kit, but can be also purchased separately, if higher volumes of solution are required.
Stop Solution (REF 00-5007-01)	
Other required Accessories/Products	
RAPTOR SERVER Analysis Software (REF 22-0000-01)	Software for the acquisition and analysis of images taken by the ImageXplorer or MAX devices
PC or Laptop	-
Pipettes & Tips	10 - 100 µl & 100 - 1000 µl
Demineralized Water	-

XII. HANDLING OF 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 ALEX³ images before array is completely dry (dry at room temperature).



XIII. WARNINGS AND PRECAUTIONS

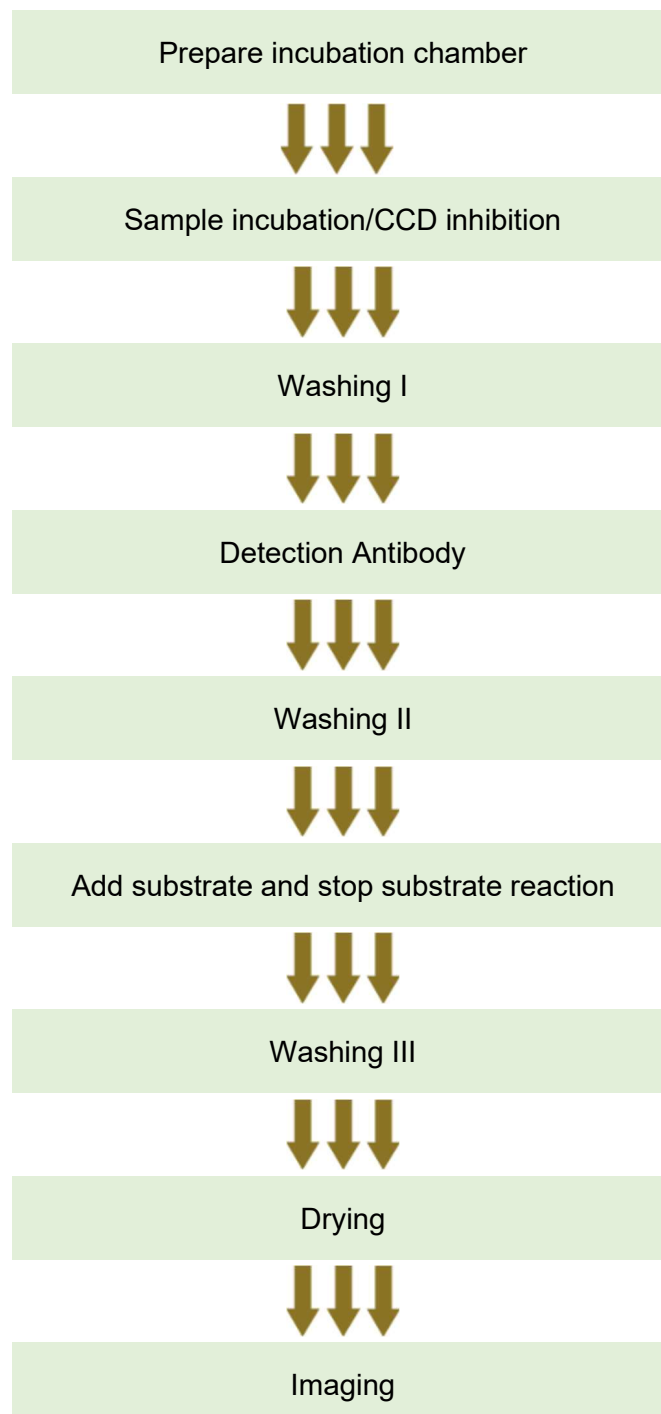
- It is recommended to wear hand and eye protection as well as lab coats and follow good laboratory practices when preparing and handling reagents and samples.
- In accordance with good laboratory practice, all blood source material (e.g. ingredients in reagents or other components) should be considered potentially infectious and handled with the same precautions as blood samples.
- ALEX³ Sample Diluent and Washing Solution contain sodium azide (<0.1%) as a preservative and must be handled with care. Safety data sheet is available upon request.
- The (ALEX³) Stop Solution contains Ethylenediaminetetraacetic acid (EDTA)-Solution 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@madx.com) or your local distributor. 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.
- ALEX³ cartridges are intended for **single use only** and must not be reused. Reagents may be opened and reused within a period of up to 6 months from the date of first opening or until the kit contents are fully consumed, whichever occurs first.
- For detailed instructions on the safe disposal of the device, refer to Chapter VIII of the IFU.
- The following P and H statements are applicable to the Stop Solution: H319 - Causes serious eye irritation; P264 - Wash skin intensively after use; P305 + P351 + P338 - If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing; P337 + P313 - In case of persisting eye irritation: get medical advice.
- Any **serious incident** that occurs in relation to the use of this device shall be reported to the **manufacturer** at support@madx.com and to the **competent authority** of the Member State in which the user and/or the patient is established.



XIV. ASSAY PROCEDURE

Manual Assay Procedure

ALEX³ kits can be used as a manual assay in combination with the ImageXplorer device (REF 11-0000-01).





Preparations

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 ALEX³ Sample Diluent
- 500 µl ALEX³ Detection Antibody
- 500 µl ALEX³ Substrate Solution
- 100 µl (ALEX³) Stop Solution
- 4500 µl Washing Solution

Assay time is approximately 3 h 30 min (without drying of processed array).

It is not recommended to run more assays than can be pipetted in 8 min. All incubations are performed at room temperature, 20-26°C.

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

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

Manual run execution

1. Sample incubation/CCD inhibition

Take out the needed number of ALEX³ cartridges and place them into the array holder(s). Add 400 µl of ALEX³ Sample Diluent to each cartridge. Add 100 µl patient sample to the cartridges. Ensure that the resulting solution is spread evenly. Place the cartridges in the prepared incubation chamber and put the incubation chamber with the cartridges on the lab rocker so that the cartridges rock along the long side of the cartridge. Start the serum incubation at 8 rpm for 2 hours. Close the incubation chamber before starting the lab rocker. After 2 hours, discharge the 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 samples between individual ALEX³ cartridges!

Optional or positive Hom s LF (CCD marker): With the standard CCD antibody inhibition protocol (as described in paragraph 2: sample incubation/CCD inhibition) 98% of all tested samples show a negative result for the CCD inhibition control marker Hom s LF. In case Hom s LF is positive with the standard protocol the following procedure is recommended:

Prepare a 1 ml sample tube, add 400 µl ALEX³ 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 in many, but not all, cases to a complete inhibition of CCD antibodies (negative Hom s LF).

1a. Washing I

Add 500 µl 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. Carefully wipe off remaining droplets from the cartridges using a paper towel.

Repeat this step 2 more times.

2. Add detection antibody

Add 500 µl of ALEX³ Detection Antibody to each cartridge.



Make sure that the complete array surface is covered by the ALEX³ Detection Antibody solution.

Place the cartridges into the incubation chamber on the lab rocker and incubate at 8 rpm for 30 minutes. Discharge the Detection Antibody solution into a collection container and vigorously tap the cartridges on a stack of dry paper towels. Carefully wipe off remaining droplets from the cartridges using a paper towel.

2a. Washing II

Add 500 µl 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. Carefully wipe off remaining droplets from the cartridges using a paper towel.

Repeat this step 4 more times.

3+4. Add ALEX³ Substrate Solution and stop substrate reaction

Add 500 µl of ALEX³ Substrate Solution to each cartridge. Start a timer with 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 exactly 8 minutes without shaking (**lab rocker at 0 rpm and in horizontal position**).

After exactly 8 minutes, add 100 µl of the (ALEX³) Stop Solution to all cartridges, starting with the first cartridge to assure that all arrays are incubated for the same time with the ALEX³ Substrate Solution. Carefully agitate to evenly distribute the (ALEX³) Stop Solution in the array cartridges, after the (ALEX³) Stop Solution was pipetted onto all arrays. Afterwards discharge the (ALEX³) Substrate/Stop Solution from the cartridges and vigorously tap the cartridges on a stack of dry paper towels. Carefully wipe off any remaining droplets from the cartridges using a paper towel.



The Lab Rocker must NOT SHAKE during substrate incubation!

4a. Washing III

Add 500 µl 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. Carefully wipe off any remaining droplets from the cartridges using a paper towel.

5. Image acquisition

After finishing the assay procedure, air dry the arrays at room temperature until they are completely dry (can take up to 45 min). If the arrays require an extended drying period, they should be stored in a light-protected environment until analysis.



The complete drying is essential for the sensitivity of the test. Only completely dried arrays provide an optimal signal to noise ratio.

The dried arrays are subsequently scanned with the ImageXplorer.



Automated Assay Procedure

ALEX³ kits can be used as an automated assay with the MAX 9k (REF 17-0000-01) or MAX 45k (REF 16-0000-01) systems. These IVD medical products automatically process ALEX technology-based arrays and acquire pictures of those.



Personnel must be trained in handling MAX devices (MAX 45k or MAX 9k). The current version of the MAX IFU (Systems) can be found here: <https://www.madx.com/extras>.

Preparations

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.

Preparation of Washing Solution (only for REF 03-5001-01 and REF 00-5003-01 when used with MAX device): Pour the content of 1 vial of Washing Solution into the washing container of the instrument. Fill demineralized water up to the red mark and carefully mix the container several times without generating foam. The opened reagent is stable for 6 months at 2-8°C.

Automated run execution

Instructions on how to run a test are provided in the MAX IFU subchapters XVII.7-10 and must be followed.

Depending on the sample volume, two operation modes are available for using ALEX³: Prediluted manually and not prediluted.



Item	MAX 45k	MAX 9k
Low volume sample tubes	Sarstedt false-bottom tubes 2.5 ml, 75x13 (round) Order number: 60.614.010	
Minimum sample volume for 1 ALEX ³ test, Low Volume tube	Not prediluted: 200 µl Prediluted manually: 120 µl serum + 480 µl Sample Diluent	
13 mm Standard tubes	Height: 75-100 mm Example: Sarstedt tubes 5 ml 75x13 mm PS Order No.: 55.475 Use with provided adapter	Height: 75mm Example: Sarstedt tubes 5ml 75x13mm PS Order No.: 55.475
16 mm Standard tubes	Minimum height: 75 mm, maximum height: 100 mm Example: Sarstedt tubes 13 ml 100x16 mm PS Order No.: 55.459	-
Minimum sample volume for 1 ALEX ³ test, 13/16 mm standard tube	Not prediluted: 400 µl Prediluted manually: 145 µl serum + 580 µl Sample Diluent	

	Before using any other tube than specified above, please contact MADx support or your distributor.
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Detailed tube requirements and instructions for dilutions are available in the MAX IFU chapter XXI (Technical Specification). The current version of the MAX IFU (Systems) can be found here: <https://www.madx.com/extras>.

Optional or positive Hom s LF (CCD marker): With the standard CCD antibody inhibition protocol (as described in paragraph 2: sample incubation/CCD inhibition) 98% of all tested samples show a negative result for the CCD inhibition control marker Hom s LF.

In case Hom s LF is positive with the standard protocol, serum samples should be pre-diluted manually (see table above) and incubated for 30 minutes (no-shaking) before loading the sample tubes into the sample carousel.

Image Analysis

Scanned ALEX-technology-based arrays are analyzed with RAPTOR SERVER Analysis Software (see details in the RAPTOR SERVER IFU at <https://www.madx.com/extras>).

The RAPTOR SERVER Analysis Software is only verified in combination with the ImageXplorer instrument and the MAX devices, therefore MADx does not take any responsibilities for results, which have been obtained with any other image capture device (like scanners).



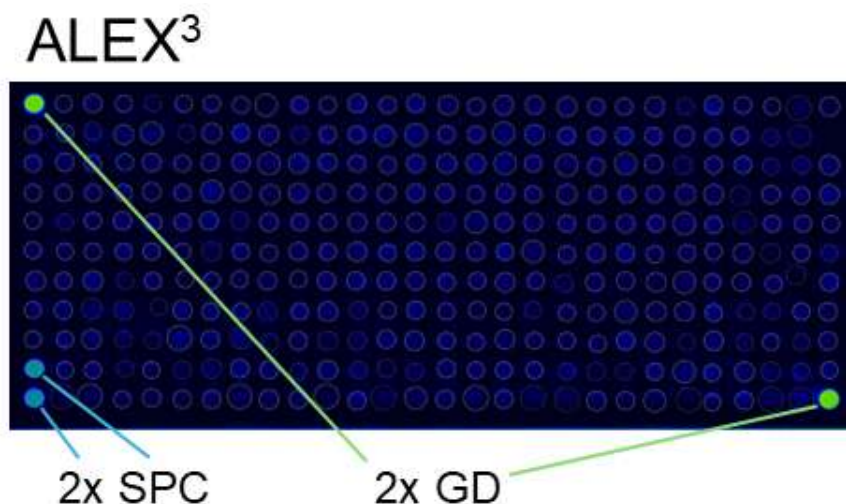
There are two Sample Pipetting Control (SPC) dots on ALEX³. Both dots are present to ensure that patient samples have been transferred to each array of the respective assay run. After processing, the SPC dots must be easily visible with the naked eye. If they are not visible, please contact your local distributor or MADx support on how to proceed. In case the SPC dots are visible, the patient samples have been pipetted correctly.

The array of the measurement with grid is displayed in the analytical image area. The software automatically identifies the position of the array in the image data based on the Guide Dots (GD). There are 2 Guide Dots on ALEX³.

After processing, the Guide Dots must be easily visible with the naked eye. Please also verify their correct orientation as shown in the image for ALEX³ below. If they are not visible, please contact your local distributor or MADx support on how to proceed. In case the Guide Dots are visible, the cartridge can be further analyzed.

During the image acquisition of an ALEX³ cartridge, RAPTOR SERVER evaluates the signal of all Guide Dots as well as the background signal of the membrane surface. If all quality criteria are fulfilled, the “automatic QC” field under the image is set to “OK”.

In order to exclude the influence of artifacts in the automated image analysis (satellite spots, sample contaminations, dust, smeared spots, ...), the images must be checked by a trained operator before the results are approved in order to exclude false results. In case of discrepancies between the processed array and the image acquired by the RAPTOR SERVER please consult your local distributor or MADx Support.



Assay Calibration

The ALEX³ master calibration curve was established by reference testing against serum preparations with specific IgE (sIgE) against different antigens covering the intended measuring range. Curve parameters for each lot are adjusted by an in-house reference testing system, against serum preparations tested with ImmunoCAP (Thermo Fisher), which is currently perceived as “reference method” for in vitro IgE testing (WAO position paper). No



reference material for sIgE is available. For tIgE, a WHO reference preparation 11/234 is available and was used for the generation of the tIgE master curve.

The sIgE results are quantitative and reported in kU_A/l . Total IgE (tIgE) results are reported in IgE response units (kU/l), where 1 kU/l correlates to 2.42 ng/ml. Total IgE results from 2-1000 kU/l are quantitative and calculated from anti-IgE measurements with lot-specific calibration factors, which are provided by the RAPTOR SERVER Analysis Software and selected according to the lot-specific QR-codes. For tIgE, results from 1001-2500 kU/l are semi-quantitative.

Systematic variations in signal levels between lots are normalized by heterologous calibration against an IgE reference curve. A correction factor is used to systematically adjust for lot-specific measurement deviations.

XV. 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. Reference values for certain commercially available control sera can be provided by MADx upon request.

XVI. DATA ANALYSIS

For the image analysis of processed arrays, the ImageXplorer or a MAX device is to be used. ALEX³ images are automatically analyzed using RAPTOR SERVER Analysis Software and a report is generated summarizing the results for the user.

XVII. RESULTS

ALEX³ quantitatively detects sIgE (in the range of 0.3-50 kU_A/l) and tIgE (in the range of 2-1000 kU/l). For tIgE levels between 1001-2500 kU/l , the detection is semi-quantitative.

Allergen-specific IgE antibodies are expressed as IgE response units (kU_A/l), total IgE results as kU/l . RAPTOR SERVER Analysis Software automatically calculates and reports sIgE and tIgE results.



Interpretation of results

The results of the ALEX³ test are automatically analyzed and presented in a report generated by the RAPTOR SERVER Analysis Software. To further support the clinical interpretation of test outcomes, a specialized software module - RAVEN Interpretation Guidance - has been implemented. **Details on the generation of the RAPTOR SERVER report and interpretation by RAVEN are provided in the RAPTOR SERVER Analysis Software IFU, subchapters VII.2.1-2.4.**

The current version of the RAPTOR SERVER Analysis Software IFU can be found at: <https://www.madx.com/extras>

XVIII. 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 on 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.

In children, especially up to 2 years of age, the normal range of tIgE is lower than in adolescents and adults [10]. Therefore, it is to be expected that in a higher proportion of children younger than 2 years the total IgE-level lies below the specified detection limit. This limitation does not apply to specific IgE measurement.

XIX. 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 ALEX³. 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 ALEX³. The reference area for total IgE in adults is < 100 kU/l. Good laboratory practice recommends that each laboratory establishes its own range of expected values.



XX. PERFORMANCE CHARACTERISTICS

The Summary of Safety and Performance can be found on the MADx website: <https://www.madx.com/extras>.

1. Accuracy

ALEX³ demonstrated high accuracy as shown by good precision (repeatability CV 6.6%, total precision CV 13.8%) and high trueness with the reference method ($R^2 > 0.9$).

(a) Precision

The precision of the ALEX³ test was evaluated according to the “single-site study” design outlined in guideline EP05-A3. Here, multiple samples are assayed on 20 days, with one run per day, and three replicates per run (“20 x 1 x 3 design”). The statistical analysis treats the sources of variation associated with “days” and “runs” as factors. This method yields estimates for two precision types: repeatability (= within-run precision) and within-laboratory precision (= within-device precision = total precision). [11]

Repeatability	Results
CV [0.3 - 1.0 kU _A /l]	13.7 %
CV [1.0 - 10.0 kU _A /l]	7.0 %
CV [>10.0 kU _A /l]	5.7 %
CV [>1.0 kU _A /l]	6.6 %
Total Precision	Results
CV [0.3 - 1.0 kU _A /l]	25.7 %
CV [1.0 - 10.0 kU _A /l]	14.4 %
CV [>10.0 kU _A /l]	12.6 %
CV [>1.0 kU _A /l]	13.8 %

(b1) Trueness of measurement (slgE)

63 different samples were tested with all 3 ALEX³ methods and ALEX² (MAX 45k only). Selected samples were also tested with the ImmunoCAP method for the priority allergens. The observed coefficient of determination, R^2 , between the different methods are shown for all priority allergens:



Ara h 2

R ²	ImmunoCAP	ALEX3 MAX 45K	ALEX3 MAX 9K	ALEX3 IX Manual	ALEX2 MAX 45k
ImmunoCAP		0,940	0,922	0,939	0,960
ALEX3 MAX 45K			0,993	0,990	0,998
ALEX3 MAX 9K				0,985	0,989
ALEX3 IX Manual					0,988
ALEX2 MAX 45k					

Phl p 1

R ²	ImmunoCAP	ALEX3 MAX 45K	ALEX3 MAX 9K	ALEX3 IX Manual	ALEX2 MAX 45k
ImmunoCAP		0,934	0,984	0,910	0,952
ALEX3 MAX 45K			0,976	0,769	0,964
ALEX3 MAX 9K				0,853	0,967
ALEX3 IX Manual					0,868
ALEX2 MAX 45k					

Gal d 1

R ²	ImmunoCAP	ALEX3 MAX 45K	ALEX3 MAX 9K	ALEX3 IX Manual	ALEX2 MAX 45k
ImmunoCAP		0,986	0,983	0,980	0,979
ALEX3 MAX 45K			1,000	0,997	0,998
ALEX3 MAX 9K				0,999	0,998
ALEX3 IX Manual					1,000
ALEX2 MAX 45k					

Pru p 3

R ²	ImmunoCAP	ALEX3 MAX 45K	ALEX3 MAX 9K	ALEX3 IX Manual	ALEX2 MAX 45k
ImmunoCAP		0,954	0,991	0,972	0,975
ALEX3 MAX 45K			0,962	0,935	0,998
ALEX3 MAX 9K				0,991	0,994
ALEX3 IX Manual					0,995
ALEX2 MAX 45k					

Art v 1

R ²	ImmunoCAP	ALEX3 MAX 45K	ALEX3 MAX 9K	ALEX3 IX Manual	ALEX2 MAX 45k
ImmunoCAP		0,949	0,952	0,940	0,951
ALEX3 MAX 45K			0,998	0,995	0,999
ALEX3 MAX 9K				0,992	0,998
ALEX3 IX Manual					0,995
ALEX2 MAX 45k					

Fel d 1

R ²	ImmunoCAP	ALEX3 MAX 45K	ALEX3 MAX 9K	ALEX3 IX Manual	ALEX2 MAX 45k
ImmunoCAP		0,911	0,954	0,844	0,871
ALEX3 MAX 45K			0,982	0,976	0,988
ALEX3 MAX 9K				0,961	0,975
ALEX3 IX Manual					0,997
ALEX2 MAX 45k					

Pla t 1

R ²	ImmunoCAP	ALEX3 MAX 45K	ALEX3 MAX 9K	ALEX3 IX Manual	ALEX2 MAX 45k
ImmunoCAP		0,949	0,993	0,987	0,978
ALEX3 MAX 45K			0,950	0,967	0,984
ALEX3 MAX 9K				0,976	0,969
ALEX3 IX Manual					0,996
ALEX2 MAX 45k					

Der p 2

R ²	ImmunoCAP	ALEX3 MAX 45K	ALEX3 MAX 9K	ALEX3 IX Manual	ALEX2 MAX 45k
ImmunoCAP		0,959	0,956	0,943	0,962
ALEX3 MAX 45K			0,991	0,983	0,993
ALEX3 MAX 9K				0,992	0,998
ALEX3 IX Manual					0,985
ALEX2 MAX 45k					

Gal d 2

R ²	ImmunoCAP	ALEX3 MAX 45K	ALEX3 MAX 9K	ALEX3 IX Manual	ALEX2 MAX 45k
ImmunoCAP		0,984	0,993	0,988	0,991
ALEX3 MAX 45K			0,997	0,999	0,999
ALEX3 MAX 9K				0,998	1,000
ALEX3 IX Manual					1,000
ALEX2 MAX 45k					

Gly m 4

R ²	ImmunoCAP	ALEX3 MAX 45K	ALEX3 MAX 9K	ALEX3 IX Manual	ALEX2 MAX 45k
ImmunoCAP		0,927	0,966	0,932	0,966
ALEX3 MAX 45K			0,992	1,000	0,992
ALEX3 MAX 9K				0,994	1,000
ALEX3 IX Manual					0,994
ALEX2 MAX 45k					

Car i

R ²	ImmunoCAP	ALEX3 MAX 45K	ALEX3 MAX 9K	ALEX3 IX Manual	ALEX2 MAX 45k
ImmunoCAP		0,899	0,942	0,935	0,947
ALEX3 MAX 45K			0,989	0,996	0,979
ALEX3 MAX 9K				0,997	0,998
ALEX3 IX Manual					0,990
ALEX2 MAX 45k					

Art v

R ²	ImmunoCAP	ALEX3 MAX 45K	ALEX3 MAX 9K	ALEX3 IX Manual	ALEX2 MAX 45k
ImmunoCAP		0,992	0,998	0,994	0,991
ALEX3 MAX 45K			0,994	0,999	0,978
ALEX3 MAX 9K				0,996	0,995
ALEX3 IX Manual					0,984
ALEX2 MAX 45k					

Results

The mean R² between all methods is **0.972**
 The mean R² between ImmunoCAP and ALEX³_MAX 45k is **0.949**
 The mean R² between ImmunoCAP and ALEX³_MAX 9k is **0.969**
 The mean R² between ImmunoCAP and ALEX³_ImageXplorer is **0.947** (for details see figure above)

(b2) Trueness of measurement (tlgE)

28 samples were tested with all 3 ALEX³ methods and ImmunoCAP as a reference method for tlgE. Additionally, a negative sample was spiked with the WHO standard 11/234 and tested in duplicates at 9 different dilutions.

Results

tlgE method comparison "real samples":
 R² tlgE ImmunoCAP and ALEX³_MAX 45k is **0.963**
 R² tlgE ImmunoCAP and ALEX³_MAX 9k is **0.919**
 R² tlgE ImmunoCAP and ALEX³_ImageXplorer is **0.963**

tlgE method comparison "spiked samples":
 R² MAX 45k is **0.996** & interdilutional CV: **5.62 %**
 R² MAX 9k is **0.981** & interdilutional CV: **5.67 %**
 R² ImageXplorer is **0.966** & interdilutional CV: **7.19 %**



4. Analytical sensitivity

The Limit of Detection (LoD) for sIgE was determined for representative allergen components (priority allergens) and is 0.3 kU_A/l for all tested allergen components. The Limit of Detection (LoD) for tIgE is 1.27 kU/l. [12]

5. Analytical specificity

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

There is no detectable interference with citrate or heparin from specimen preparation nor with bilirubin, cholesterol/triglycerides and hemoglobin at normal physiological concentrations. Neither is there an interference with tIgE, which was tested in concentrations of up to 3000 kU/l.

6. Metrological traceability of calibrator and control material values

N/A

7. Measuring range of the assay

The measuring range was set as follows:

Specific IgE (quantitative): 0.3-50 kU_A/l

Total IgE (quantitative): 2-1000 kU/l

Total IgE (semi-quantitative): 1001-2500 kU/l

This is based on the LoD and the upper measuring range limit (hook effect), which was calculated in general alignment with CLSI guideline I/LA20, 3rd ed.

The Assay Cut-Off is 0.3 kU_A/l for specific IgE.

(a) Linearity

Positive serum samples were serially diluted with blank sample to 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and 1:128. Undiluted and diluted samples were tested according to the ALEX³ assay protocol.

For total IgE, a blank sample was spiked with the WHO preparation 11/234 as the 3rd International Standard for serum IgE at a concentration of 2500 kU/l and serially diluted to cover the full measuring range.



The obtained values (O) are compared with the estimated values (E). The quotient O/E is calculated for the selected priority allergens.

Results	<u>slgE:</u> 83.8% (or 264 out of 315) of all O/E values for the priority allergens were within 0.8 -1.2
	<u>tlgE:</u> 85.0% (or 17 out of 20) of all O/E values of the spiked tlgE preparations were within 0.8 -1.2

8. Information on clinical performance

Since 247 of 300 allergens were already present on the ALEX² array, the clinical performance evaluation of ALEX² is in part applicable to ALEX³. 53 new allergens were validated by using sera from clinically well-characterized patients, sensitized to the respective allergen source. All allergens were evaluated with clinically validated sera. The new allergens were shown to specifically bind IgE antibodies from at least five patients sensitized to the corresponding allergen source. Where applicable, the results were also compared to the dedicated ImmunoCAP specific allergens, further confirming the specificity. The specificity of the new allergens was validated by the absence of IgE binding in sera from non-sensitized individuals. In cases where a specific ImmunoCAP was not available, the allergen extract was effectively used as a surrogate, demonstrating the robustness of the clinical performance. The clinical performance is therefore verified.

(a) Clinical Study ALEX²

A clinical study titled "Diagnostic Accuracy of the MADx Multi Array Xplorer (MAX 45k) Automated Laboratory System and the MADx Allergy Explorer Version 2 (ALEX²) – IgE Multiplex Test for the Diagnosis of Pre-defined Groups of Specific High-priority Allergens (MADMAX)" (ClinicalTrials.gov ID: NCT04435678) was successfully completed in April 2022.

A total of 837 patients were enrolled, including 689 allergic and 148 non-allergic individuals.

(a1) Sensitivity:

Allergy Type	Sensitivity (95% Confidence interval)
Birch pollen allergy (n=111)	94.6% (88.6-98.0)
Grass pollen allergy (n=113)	98.2% (93.8-99.8)
House dust mite allergy (n=148)	91.2% (85.4-95.2)
Cat allergy (n=107)	92.5% (85.8-96.7)
Bee venom allergy (n=104)	76.0% (66.6-83.8)
Vespid venom allergy (n=106)	94.3% (88.1-97.9)

**(a2) Specificity:**

Test	Specificity (95% Confidence interval)
ALEX ² test compared to clinical symptoms (n=146)	95.9% (91.3-98.5)

(a3) Likelihood Ratio

The likelihood ratio was calculated from the sensitivity and specificity data according to the following formula: Sensitivity / (1 - Specificity).

Allergy Type	Calculated Likelihood ratio
Birch pollen allergy (n=111)	23.1 (0.946/(1-0.959))
Grass pollen allergy (n=113)	24.0 (0.982/(1-0.959))
House dust mite allergy (n=148)	22.2 (0.912/(1-0.959))
Cat allergy (n=107)	22.6 (0.925/(1-0.959))
Bee venom allergy (n=104)	18.5 (0.760/(1-0.959))
Vespid venom allergy (n=106)	23.0 (0.943/(1-0.959))

9. Information on stability

Stability testing in an accelerated stability study of ALEX³ showed high robustness, 2 years post-production stored at 2-8°C. Thus, the determined shelf life is 2 years. Additionally, as part of the accelerated stability study, transport stability studies were performed. For the transport simulation study, the kits were subjected to a transport simulation (TS) protocol before they were tested. Additionally, the packaging and labelling was tested for their convenience.

XXI. WARRANTY

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



XXII. ABBREVIATIONS

ALEX	Allergy Xplorer
CCD	Cross-reactive carbohydrate determinants
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
IgE	Immunoglobulin E
IVD	In-vitro diagnostic
kU/l	Kilo units per Liter
kU _A /l	Kilo units of allergen-specific IgE per liter
MADx	MacroArray Diagnostics
REF	Reference number
rpm	Rounds per minute
sIgE	Allergen-specific IgE
tIgE	Total IgE
µl	Microliter



ALLERGEN LIST ALEX³

Allergen extracts:

Aca m, Aca s, Ach d, Ail a, All c, All s, Ama r, Amb a, Api m, Art v, Ave s, Ber e, Bos d_meat, Bos d_milk, Bro pa, Cam d, Can f_male urine, Can s, Cap h_epithelia, Cap h_milk, Car i, Car p, Che a, Che q, Chi spp, Cic a, Cla h, Clu h, Cuc p, Cup s, Equ c_meat, Equ c_milk, Fag e, Fic b, Fic c, Gal d_meat, Gal d_white, Gal d_yolk, Hel a, Hom g, Hor v, Jug r_pollen, Jun a, Lit spp, Loc m, Lol spp, Lup a, Mac i, Mel g, Ory_meat, Ovi a_meat, Ovi a_milk, Pan b, Pan m, Pap s, Par j, Pas n, Pen ch, Per a, Pers a, Phr c, Pin p, Pol d, Pru du, Pyr c, Raj c, Rud spp, Sal k, Sal s, Sco s, Sec c_flour, Sec c_pollen, Ses i, Sin a, Sol spp, Sol t, Sola l, Sus d_epithelia, Ten m, Tri s, Tyr p, Zea m

Purified natural components:

α -Gal, nAct d1 nAna o 1, nAna o 3, nApi m 1, nAra h 1, nAra h 3, nBos d 12, nBos d 4, nBos d 5, nBos d 6, nBos d 8, nCan f 3, nCoc n 1, nCor a 11, nCor a 9, nCry j 1, nCup a 1, nEqu c 3, nFag e 2, nGal d 2, nGal d 3, nGal d 4, nGal d 5, nGly m 5, nGly m 6, nJug r 4, nLit v 7, nMac i 1.0101(28-76), nOle e 7, nPap s 1.0101 (27-846), nPis v 2, nPis v 3, nPla a 2, nPru du 6, nSal s 6, nTri a aA_Tl, nZea m 1

Recombinant components:

rAct d 10, rAct d 2, rAct d 5, rAln g 1, rAln g 4, rAlt a 1, rAlt a 6, rAmb a 1, rAmb a 4, rAna o 2, rAni s 1, rAni s 3, rApi g 1, rApi g 2, rApi g 6, rApi g 7, rApi m 10, rApi m 2, rAra h 15, rAra h 18, rAra h 2, rAra h 6, rAra h 8, rAra h 9, rArg r 1, rArt v 1.0101, rArt v 3.0201, rAsp f 1, rAsp f 3, rAsp f 4, rAsp f 6, rAsp f 8, rBer e 1, rBet v 1, rBet v 6, rBet v 7, rBla g 1, rBla g 2, rBla g 4, rBla g 5, rBla g 9, rBlo t 10, rBlo t 2, rBlo t 21, rBlo t 5, rBos d 10, rBos d 11, rBos d 2, rBos d 9, rCan f 1, rCan f 2, rCan f 4, rCan f 6, rCan f Fel d 1 like, rCan s 3, rCar i 1, rCari 2 (256-386), rCar i 4, rCav p 1, rChe a 1, rCla h 8, rClu h 1, rCor a 1.0401, rCor a 14, rCor a 8, rCra c 6, rCuc m 2, rCyn d 1, rCyp c 1, rCyp c 2, rDer f 1, rDer f 15, rDer f 18, rDer f 2, rDer p 1, rDer p 10, rDer p 2, rDer p 20, rDer p 21, rDer p 23, rDer p 5, rDer p 7, rDol m 2, rDol m 5, rEqu c 1, rEqu c 4, rFel d 1, rFel d 2, rFel d 4, rFel d 7, rFra a 3, rFra e 1, rGad m 1, rGal d 1, rGal d 7, rGly d 2, rGly m 4, rGly m 8, rHel a 3, rHev b 1, rHev b 11, rHev b 3, rHev b 5, rHev b 6.02, rHom s LF, rJug r 1, rJug r 2, rJug r 3, rJug r 6, rLen c 1, rLen c 3, rLep d 2, rMac r 1, rMac r 2, rMal d 1, rMal d 3, rMala s 11, rMala s 13, rMala s 5, rMala s 6, rMan i 1, rMes a 1, rMus a 2, rMus a 5, rMus m 1, rOle e 1, rOle e 9, rOry c 1, rOry c 2, rOry c 3, rPar j 2, rPen m 1, rPen m 2, rPen m 3, rPen m 4, rPer a 6, rPer a 7, rPers a 1, rPhl p 1, rPhl p 12, rPhl p 2, rPhl p 5.0101, rPhl p 6, rPhl p 7, rPhod s 1, rPin p 1, rPis s 1, rPis s 2, rPis s 3, rPis v 1, rPla a 1, rPla a 3, rPla l 1, rPol d 5, rPru av 3, rPru p 3, rPru p 7, rQue a 1, rRaj c Parvalbumin, rRat n 1, rSal k 1, rSal k 5, rSal s 1, rSco s 1, rSes i 1, rSin a 1, rSola l 6, rSus d 1, rThu a 1, rTri a 14, rTri a 19, rTri a 36, rTri a 37, rTyr p 10, rTyr p 2, rVes v 1, rVes v 5, rVit v 1, rXip g 1, rZea m 14



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CHANGE HISTORY

Version	Description	Date of Issue	Replaces
03	Title was changed to ALEX ³ Allergy Xplorer; Note on manual versus automated assay was added to chapter III; Information on shipment and storage of samples was included in chapter VII; Information on safe disposal of the device was added to chapter VIII; New label icon was added to chapter IX; Information on new reagent nomenclature and reactive ingredients of kit components was added to chapter X; Table with required equipment for automated and manual processing was included in chapter XI; Note on single use, P and H statements and serious incident reporting was added to chapter XIII; Chapter XIV was changed to 'Assay Procedure'; Manual and automated assay description was adapted in chapter XIV to better emphasize the differences; Information on interpretation of results was included in chapter XVII; Note on Accuracy, Metrological traceability, Measuring range of the assay and Clinical performance was added to chapter XX; Date of Issue added to Change History.	08-2025	02



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