IMUNO DIAGNOSTIKA

AllergyCHIP – quantitative detection of allergen-specific IgE
Instructions for use

The kit is intended for in vitro diagnostics IVD

Catalogue No.	Specific antibodies	Ig class	Substrate	Format
SC-2022 E	**Allergen specific IgE**	**IgE**	**Antigen – coated solid phase (microarray)**	**2 x 21 (chip x reaction sites)**

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1. PURPOSE OF USE

AllergyCHIP is an in vitro quantitative assay for the measurement of allergen specific IgE antibodies in human serum or plasma. It is intended for in vitro diagnostic use as an aid in the clinical diagnosis of IgE mediated allergic disorders in conjunction with other clinical findings. It is to be used by clinical laboratories and well trained laboratory specialists. This product is designed for diagnostics IVD and support the clinical diagnosis of specific IgE mediated diseases alongside other clinical findings.

2. 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, hay fever, atopic eczema and gastro intestinal symptoms.

3. TEST PRINCIPLE

AllergyCHIP is a solid-phase immunoassay. Allergens extracts or allergen components that are immobilized on a solid substrate in a microarray format react with the specific IgE in the patient sample. After incubation, non-specific IgE is washed off. The procedure continues by adding a fluorescence-labeled anti-human IgE antibody to form a complex. After incubation unbound fluorescence labeled anti-human IgE antibodies are removed by second washing. The procedure is followed by fluorescence measurement using an appropriate microarray scanner. The higher the response value the more specific IgE is present in the specimen. The test results are analyzed with microarray image analysis software MAPIX and reported in IgE response units (kU_A/L).

4. REAGENTS 2 x 21

Components	*Content*	*Properties*
AllergyCHIP	2 chips with 21 reaction sites each	Ready for use. Store at 2-8°C until expiration date. Allow to reach room temperature before opening the vacuum seal. After the vacuum seal is broken unused chips can be stored at 2-8°C in the desiccant containing, resealed foil bag.
Dilution buffer	1 × 8 ml	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.
Wash buffer, 10x concentrated	1 × 50 ml	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.

Positive control (PC) Sodium azide < 0,1% (human serum)	1 × 200 µl	Ready for use. Store at 2-8°C until expiration date. Do not dilute. Opened vial can be stored at 2-8°C for 6 months if recapped.
Detection Antibody (Fluorescence conjugated anti-human IgE) Sodium azide < 0,1%	1 × 4 ml	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.
Protective seal	4 pcs	-
Instructions for use	2 pcs	-

5. ADDITIONAL MATERIALS AND EQUIPMENT(not included into the package)

- Microarray scanner
- Calibrated pipettes
- Microchip holder for incubations
- Microchip washing dish with holding rack (2 pcs.)
- Washing reagent reservoirs (2 pcs.)
- Pipette tips
- Plastic tubes or 96 well plates for sample dilutions
- Multichannel pipette
- Magnetic stirrer
- Measuring cylinder 1000 ml
- Distilled or deionized water
- PC/Laptop
- Microplate incubator – for incubation at +37°C
- The incubator or a water bath is recommended for heating the wash buffer if needed
- Watch or a stopwatch

6. STORAGE AND STABILITY

The package contents must be stored at temperature between 2°C and 8 °C. **Important!** Do not freeze.
As long as the packaged is sealed, all test reagents remain stable until the date of expiry, indicated on the package.
After opening, when stored at 2 to 8 °C and tightly closed, they remain stable until the date of expiry, indicated on the package, unless indicated otherwise in the instructions below.

7. WARNINGS AND SAFETY PRECAUTIONS

- The product must be used only by trained clinical research laboratory staff.
- If the packaged reagents are visibly damaged, do not use the kit.
- Read the instructions for use carefully before use. Use only the instructions, supplied together with the product in the same package.
- When performing the test, follow the volumes, incubation time, temperature and preparation stage, indicated in the instructions.
- Do not change or mix reagents with reagents of other manufacturers.
- Observe Good Laboratory Practice (GLP) and work safety requirements. Some of the reagents contain undeclared amounts of preservatives. Avoid the sample and reagent contact with eyes and skin. In case of

contact with eyes or skin, wash thoroughly with water. Change or wash contaminated clothes. In case of swallowing, immediately seek for medical help.

- Positive control contain biological samples of human origin. Appropriate safety measures are recommended for working with these controls, the same as with blood samples.

8. SAMPLE PREPARATION AND STABILITY

Human serum or plasma with EDTA samples from venous or capillary blood can be used and must be diluted with the dilution buffer at the ratio of 1:4. For example, 30 µl sample must be diluted with 120 µl of dilution buffer.

Collect blood samples using standard procedures. Keep serum at room temperature for shipping purposes only, for maximum 120 hours from the time the blood was taken. The samples can be stored at2 °C to 8 °C for up to 7 days. Keep serum and plasma samples at -20°C for prolonged storage. Avoid repeated freezing and thawing. Allow samples to reach room temperature before use. It is recommended to dilute and use the samples on the day of conducting the testing.

9. REAGENT PREPARATION AND STABILITY

Note: all reagents must be taken out and kept at room temperature (+20°C to +25 °C) at least for 30 min.
Set the incubator for the chip incubation at +37 °C.

- **AllergyCHIP** Ready for use. Before opening the protective package of the chip, the chip must be kept at room temperature for minimum 30 min. (Thus, preventing the accumulation of moisture (condensate)). Do not label with pens or markers that are soluble in water or organic solvents. Residuals of labeling can interfere with the fluorescence-based analysis of AllergyCHIP. If necessary, use a pencil for labeling. Avoid direct contact with the surface of the chip during any of the reaction steps. Always handle the chip by grasping the edge of the glass. Do not acquire AllergyCHIP images before array is completely dry.
- **Positive control (PC)**. Mix the reagent well before use. Ready for use. Store at 2-8°C until expiration date. Do not dilute. Opened vial can be stored at 2-8°C for 6 months if recapped.
- **Detection Antibody.** 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.
- **Dilution buffer.** 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
- **Wash buffer.** The wash buffer is a 10x concentrate. In case of visually visible crystallisation in the concentrated wash buffer, heat it to +37 °C and mix well before dilution. The concentrate must be diluted with distilled/deionized water (dH_2O) at 1:9 (for example by adding 25 ml of wash buffer concentrate into 225 ml of dH_2O). **Note:** Ready for use wash solution remains stable storing at 2 to 8 °C for 4 weeks.

10. WASTE MANAGEMENT

Patient samples, positive control and AllergyCHIP must be managed as possible sources of infection. All reagents must be managed and disposed of in accordance with local laboratory waste management regulations.

11. ASSAY PROCEDURE

1 Step 1

11.1. Incubation with tested sample (serum or plasma)

Add **80 µl** of positive control and prepared diluted patient samples (see pt. 8) into AllergyCHIP wells. Ensure that the entire reaction site is covered with sample. Avoid direct contact of the pipette tip with the surface of the chip when dispensing the sample. Cover the prepared microarray with a protective seal and incubate at +37 °C for 2 hours. The example, AllergyCHIP filling scheme for testing 20 samples is provided in the table:

1	2	3
4	5	6
7	8	9
10	11	12
13	14	15
16	17	18
19	20	PC
Barcode		

Scheme description: testing of 20 samples. Positive control (PC) is internal quality control of the test and is needed to assess the reliability of the test conducted. This is mandatory upon conducting each test.

11.2. Washing

Remove protective seal, empty the content, subsequently wash the microplate 5 times (5 washing cycles) by adding **100 µl** of ready to use wash buffer (see pt. 9). Empty out the content. The remainder of the wash solution must be removed by turning the microchip holder upside-down and shaking out the remaining wash buffer. The washing can be done using an automatic device.

Step 2

11.3. Incubation with Detection Antibody

Fill each AllergyCHIP field with **80 µl** of ready to use (see pt. 9) Detection Antibody. Cover the microplate with a protective seal and incubate at +37 °C for 30 min.

11.4. Washing

Remove the protective seal. Subsequently wash the microplate 5 times (5 washing cycles) by adding **100 µl** of ready to use wash solution (see pt. 9). Empty out the content. The remainder of the wash solution must be removed by turning the microchip holder upside-down and shaking out the remaining wash buffer.

Step 3

11.5. Final washing

Add approximately 220 ml of Washing Solution and put a magnetic stirring bar in the bottom of the Washing Dish. Take out the chips from a microchip holder and put the chips into the removable Washing Rack (up to 10 chips per rack) and place it in the Washing Dish. Place Washing Dish onto a magnetic stirrer and stir at slow to medium speed for 10 minutes. Move the Washing Rack containing the chips into a separate Washing Dish containing approximately 220 ml purified water for 30 seconds. Remove the Washing Rack containing the chips and place it onto a paper towel and leave to air-dry (approximately 15 minutes) or use glass slide centrifuge for 10 s. The chips must be completely dry. Continue with the chip scanning immediately afterwards. Discard all used washing solutions. The chips can be stored dry and protected from light for up to 1 week for later reading.

12. DATA ANALYSIS

12.1. Scanning procedure

For the analysis of AllergyCHIP a laser scanner shall be used. A scanning protocol for AllergyCHIP is set up during system installation by the technical product specialist. The MAPIX and Luxscan software are the only verified in combination with the Innopsys and LuxScan 10K-A scanner instruments, therefore Imunodiagnostika does not take any responsibilities for results, which have been obtained with any other laser scanning devices.

12.2. 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 testingagainst a serum preparation tested on ImmunoCAP (Thermo Fisher Scientific) for specific IgE against several allergens. The AllergyCHIP results are therefore indirectly traceable against the WHO reference preparation 11/234 for total IgE. AllergyCHIP sIgE test results for allergen components are expressed as kU_A/L.

13. QUALITY CONTROL

According to good laboratory practice it is recommended to record the lot numbers of all reagents used. According to good laboratory practice it is recommended that quality control samples are included within defined intervals.

14. LIMITATIONS OF THE PROCEDURE

- A definitive clinical diagnosis should not be based on the results of any single diagnostic method, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
- In food allergy, circulating IgE antibodies may remain undetectable despite a convincing clinical history because these antibodies may be directed towards allergens that are revealed or altered during industrial processing, cooking or digestion and therefore do not exist in the original food for which the patient is tested.
- Insufficient washing may increase the test values.
- The remaining washing solution in the fields may result in falsely low-test values.

15. EXPECTED VALUES

The close association between allergen specific IgE antibody levels and allergic disease is well known and is described thoroughly. Each sensitized patient will show an individual IgE profile when tested with AllergyCHIP. The IgE response with samples from healthy non-allergic individuals will be below 0.35 kU_A/L for single molecular allergens and for allergen extracts when tested with AllergyCHIP. Good laboratory practice recommends that each laboratory establishes its own range of expected values.

16. PERFORMANCE CHARACTERISTICS

16.1. Precision

The following mean coefficients of variation (CV%) have been obtained when testing representative allergen components using the same lot of reagents. 6 samples were assayed in 3 replicates on 6 different occasions:

Concentration - kU_A/L	Coefficients of Variation (CV%)	
	Within	Between
≥ 0,35 - < 3,49	19	25
≥ 3,5 - < 17,49	14	20
≥ 17,5	1	27

16.2. Analytical specificity

There is no detectable cross-reactivity with other human Immunoglobulins (IgA, IgG1, IgG2, IgG3, IgG4 and IgM) at normal physiological concentrations.16.3. Analytical Sensitivity

The Limit of Detection was determined in accordance with CLSI guideline EP17-A for allergen specific IgE antibodies was below 0.35 kU_A/below for all allergens tested.

16.4. Interference

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

17. PERFORMANCE CHARACTERISTICS

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

18. ALLERGEN LIST

Act d; Aln g; Aln g 1; Alt a 1; Amb a; Ana o; Api g; Ara h; Art v; Art v 1; Ave s; Bet v 1; Bet v 2; Bet v 4; Bos d (milk); Can f 1; Can f 2; Can f 4; Can f 5; Can f 6; Cyp c; Cor a (nut); Cor a 1.01; Cor a 1.04; Dau c 1; Der f; Der f 1; Der f 2; Der p; Der p 1; Der p 10; Der p 2; Der p 23; Equ c (epithelia); Equ c 1; Fel d (epithelia); Fel d 1; Fel d 2; Fel d 4; Fra a 1; Gad m; Gal d (egg white); Gal d 1; Gal d 2; Hel a; Hor v; Jug r (nut); Mal d; Mus m 1; Ory c (epithelia); Pen m; Phl p; Phl p 1; Phl p 11; Phl p 12; Phl p 2; Phl p 5; Phl p 7; Pis v; Pru p 1; Pru p 3; Sal s; Sol t; Sus s 1.

LEGEND

IVD	In vitro diagnostic measure		Date of manufacture
CE	CE marked		Manufacturer
LOT	Product LOT number	REF	Catalogue number
2°C 8°C	Storage temperature		Biohazard
	Expiration date		Follow the instructions for use inside
	The reagent is sensitive to light		