Instruction for use

**A SOLID-PHASE ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF *ASPERGILLUS FUMIGATUS* ALLERGEN SPECIFIC IgE IN HUMAN SERUM OR PLASMA**

# ASPERGILLUS FUMIGATUS ALLERGEN SPECIFIC IgE EIA

REF **K200Sm3**

For 96 determinations

IVD

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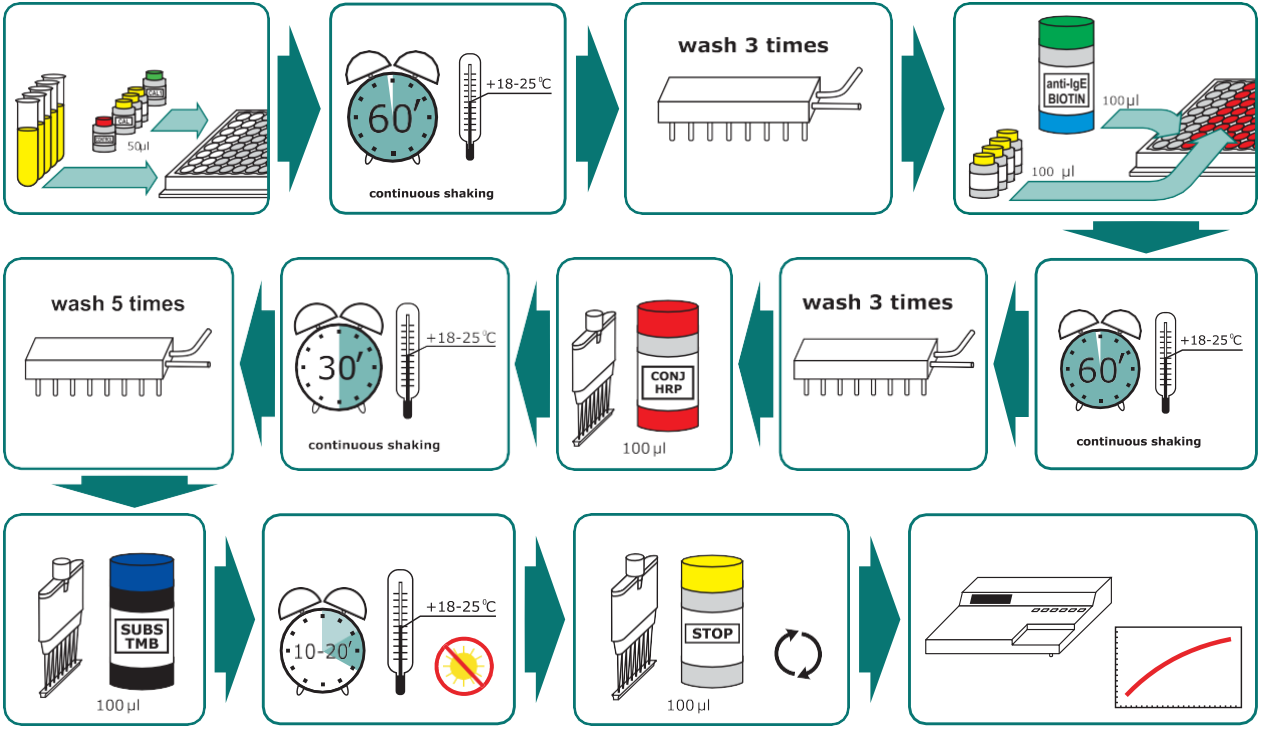
# Test procedure




**K200S**

**CONTENTS**

*Instruction for use.*

# A SOLID-PHASE ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF ASPERGILLUS FUMIGATUS ALLERGEN SPECIFIC IgE IN HUMAN SERUM OR PLASMA

## 1. INTENDED USE

A solid-phase enzyme immunoassay for the quantitative determination of allergen-specific IgE in blood serum or plasma.

This kit is designed for measurement of allergen-specific IgE in blood serum or plasma. For possibility of use with other sample types, please, refer to Application Notes (on request). The kit contains reagents sufficient for 96 determinations and allows to analyze 41 unknown samples in duplicates.

## 2. SUMMARY AND EXPLANATION

Specific IgE (sIgE) mediates type I (immediate) allergic reactions, with usual onset of symptoms (allergic rhinitis, conjunctivitis, urticaria, asthma, anaphylactic shock) within 30 minutes after exposure. If allergen-specific IgE is found, it helps to make a diagnosis of type I allergy.

## 3. PRINCIPLE OF THE TEST

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific murine monoclonal antibody to human IgE. Antigen from the specimen is captured by the antibodies coated onto the microwell surface. Unbound material is removed by washing procedure. Allergen-specific IgE bound are revealed by biotinylated allergen and streptavidin-HRPO complex. After washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450/492 nm. Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen.

## 4. WARNINGS AND PRECAUTIONS

**4.1.** For professional use only.

**4.2.** This kit is intended for in vitro diagnostic use only.

**4.3.** INFECTION HAZARD: There is no available test methods that can absolutely assure that Hepatitis B and C viruses, HIV-1/2, or other infectious agents are not present in the reagents of this kit. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

**4.4.** Avoid contact with stop solution containing 5.0% $H_2SO_4$. It may cause skin irritation and burns.

**4.5.** Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents may give false results.

**4.6.** Do not use the kit beyond the expiration date.

**4.7.** All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microplate readers.

**4.8.** Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

**4.9.** Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.

**4.10.** Do not mix reagents from different lots.

**4.11.** Replace caps on reagents immediately. Do not swap caps.

**4.12.** Do not pipette reagents by mouth.

**4.13.** Specimens must not contain any AZIDE compounds – they inhibit activity of peroxidase.

**4.14.** Material Safety Data Sheet for this product is available upon request directly from XEMA Co., Ltd.

**4.15.** The Material Safety Data Sheet fit the requirements of EU Guideline 91/155 EC.

## 5. KIT COMPONENTS

**5.1.** Contents of the Kit

| | Symbol | Description | | Qty | Units | Colour code | Stability of opened/ diluted components |
|---|---|---|---|---|---|---|---|
| 1 | SORB MTP | Specific m3 IgE EIA strips, 8x12 wells | polystyrene microwells coated with murine monoclonal antibody to human IgE | 1 | pcs | | until exp. date |
| 2 | CAL 1–6 | Calibrator set, 0.8 ml each. The set contains 6 calibrators: 0; 0.35; 0.7; 3.5; 17.5; 100 IU/ml | human IgE diluted in phosphate buffered of horse serum, casein solution, preservative - 0.1% phenol; also contains red dye | 6 | pcs | red (C1 - colourless) | 2 months |
| 3 | CONTROL | Control serum (0.8 ml) | dilution of preselected human serum, with known content of IgE with casein solution; preservative - 0.1% phenol; also contains purple dye | 1 | pcs | purple | 2 months |
| 4 | CONJ HRP | Conjugate, 14 ml | aqueous solution of streptavidin coupled with horseradish peroxidase diluted on phosphate buffered solution with casein from bovine milk and detergent (Tween-20), contains 0.1% phenol as preservative and red dye | 1 | pcs | red | until exp. date |
| 5 | SUBS TMB | Substrate solution, 14 ml | ready-to-use single-component tetramethylbenzidine (TMB) solution. | 1 | pcs | colourless | until exp. date |
| 6 | BUF WASH 26X | Washing solution concentrate 26X, 22 ml | aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative | 1 | pcs | colourless | Concentrate – until exp. date Diluted washing solution – 45 days at 2-8 °C or 15 days at RT |
| 7 | STOP | Stop solution, 14 ml | 5.0% vol/vol solution of sulphuric acid | 1 | pcs | colourless | until exp. date |
| 8 | anti-IgE BIOTIN | Biotin labeled mAb to IgE (11 мл) | aqueous solution of mAb to IgE coupled with horseradish peroxidase diluted on phosphate buffered solution with casein from bovine milk and detergent (Tween-20), contains 0,1% phenol as preservative and blue dye | 1 | pcs | blue | until exp. date |
| 9 | N003 | Plate sealing tape | | 2 | pcs | | N/A |
| 10 | K200Sm3I | Instruction Specific m3 IgE EIA | | 1 | pcs | | N/A |
| 11 | K200SQm3 | QC data sheet Specific m3 IgE EIA | | 1 | pcs | | N/A |

**Allergen-biotin**

Biotinylated allergen (ready-to-use, 10 ml, blue transparent liquids).

1. **m3** - *Aspergillus fumigatus* allergen.

**5.2.** Equipment and material required but not provided

- Distilled or deionized water;
- Automatic or semiautomatic multichannel micropipettes, 100–250 µl, is useful but not essential;
- Calibrated micropipettes with variable volume, range volume 25–250 µl;
- Microtiter plate shaker. Shaking should be medium to vigorous. Longitudinal shaking approximately 200 strokes/min, oscillations 600–800/min
- Calibrated microplate photometer with 450\492 nm wavelength and OD measuring range 0–3.0.

**5.3.** Storage and stability of the Kit

Store the whole kit at +2…+8 °C upon receipt until the expiration date.

After opening the pouch keep unused microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture.

## 6. SPECIMEN COLLECTION AND STORAGE.

This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided.

**Avoid freezing-thawing of samples!**

**Store serum/plasma samples at +2…+8 °C for nmt 7 days.**

Important notice: biotin (vitamin B7) in a sample may cause incorrect results. Patients should avoid biotin-containing food (liver, eggs, cereals (esp., soy), pea, codfish, chicken, pistachios, milk products), drugs and/or supplements during three days before testing.

## 7. TEST PROCEDURE

**7.1.** Reagent Preparation

- All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18…+25 °C) before use.
- All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.
- It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.
- Prepare washing solution from the concentrate BUF WASH 26X by 26 dilutions in distilled water.

**7.2.** Procedural Note:

It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

**7.3.** Assay flowchart

See the example of calibration graphic in Quality Control data sheet.

**7.4.** Assay procedure

| | |
|---|---|
| 1 | Put the desired number of microstrips into the frame; allocate 12 wells for the calibrators CAL 1–6 and control samples CONTROL and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS. |
| 2 | Pipet 50 µl of calibrators CAL 1–6 and unknown samples into the wells. Cover the wells by plate adhesive tape. |
| 3 | Incubate 45 minutes at room temperature +18…+25 °C . |
| 4 | Prepare washing solution by 26X dilution of washing solution concentrate BUF WASH 26X by distilled water. Minimal quantity of washing solution should be 250 µl per well. Wash strips 3 times. |
| 5 | ATTENTION! Dispense 100 µl of biotin labeled mAb to IgE solution into the wells allocated for calibrator and controls. Dispense 100 µl of biotin labeled allergen solution into the wells allocated for samples. Cover the wells by plate adhesive tape. |
| 6 | Incubate 45 minutes at room temperature +18…+25 °C . |
| 7 | Wash strips 3 times. |
| 8 | Dispense 100 µl of CONJ HRP into the wells. Cover the wells by plate adhesive tape. |
| 9 | Incubate 30 minutes at room temperature +18…+25 °C . |
| 10 | Wash the strips 5 times. |
| 11 | Dispense 100 µl of SUBS TMB into the wells. |
| 12 | Incubate 20 minutes at +18…+25 °C. |
| 13 | Dispense 100 µl of STOP into the wells. |
| 14 | Apply point-by-point method for data reduction in the range of CAL1-CAL5.<br>ATTENTION: CAL6 point should be omitted in data reduction by standard run mode!<br><br>Measure OD (optical density) at 492 nm if concentration of analyte more than CAL5 only.Set photometer blank on first calibrator. Apply point-by-point method for data reduction in the range of CAL1-CAL6.<br>ATTENTION: CAL2 point should be omitted in data reduction! |

## 8. QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state, and local standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications.

## 9. CALCULATION OF RESULTS

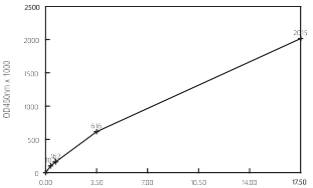
**9.1.** Calculate the mean absorbance values (OD450) for each pair of calibrators and samples.

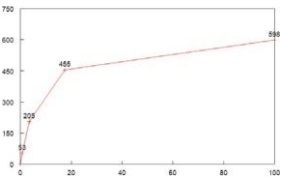
**9.2.** Plot a calibration curve on graph paper: OD versus allergen-specific IgE concentration.

**9.3.** Determine the corresponding concentration of allergen-specific IgE in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

**9.4.** Below is presented a typical example of a standard curve with the XEMA Co. Not for calculations!

| Calibrators | Value | Absorbance Units (450 nm) | Absorbance Units (492 nm) |
|---|---|---|---|
| CAL 1 | 0 IU/ml | 0.06 | 0.04 |
| CAL 3 | 0.7 IU/ml | 0.16 | 0.092 |
| CAL 4 | 3.5 IU/ml | 0.22 | 0.244 |
| CAL 5 | 17.5 IU/ml | 0.68 | 0.495 |
| CAL 6 | 100 IU/ml | 2.08 | 0.638 |



## 10. EXPECTED VALUES

NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

| RAST classes | Units, IU/ml | |
|---|---|---|
| | Lower limit | Upper limit |
| 0 - not determined | - | <0.35 |
| 1 - low | 0.35 | 0.7 |
| 2 - moderate | 0.7 | 3.5 |
| 3 - high | 3.5 | 17.5 |
| 4 - very high | 17.5 | - |

## 11. PERFORMANCE CHARACTERISTICS

**11.1.** Analytical specificity / Cross reactivity

High specificity is provided by use of mouse monoclonal antibodies to IgE.

| Analyte | Cross-reactivity, % wt/wt |
|---|---|
| IgG | <0.1 |
| IgM | <0.1 |
| IgA | <0.1 |

**11.2.** Analytical sensitivity.

Sensitivity of the assay was assessed as being 0.05 IU/ml.

**11.3.** Linearity.

Linearity was checked by assaying dilution series of 5 samples with different allergen-specific IgE concentrations. Linearity percentages obtained ranged within 90 to 110%.

**11.4.** Recovery.

Recovery was estimated by assaying 5 mixed samples with known allergen-specific IgE concentrations. The recovery percentages ranged from 90 to 110%.

| Символ / Symbol | Значение символа / Symbolize |
|---|---|
|  | Производитель / Manufacturer |
|  | Дата производства / Date of manufacture |
| REF | Номер по каталогу / Catalogue number |
| LOT | Номер серии / Batch code |
|  YYYY-MM | Использовать до (год-месяц) / Use By |
|  | Ограничение температуры / Temperature limitation |
| IVD | Только для ин витро диагностики / In Vitro Diagnostic Medical Device |
|  | Внимание! / Caution, consult accompanying documents |
|  | Не использовать при нарушении целостности упаковки / Do not use if package damaged |
| SORB MTP | Планшет / EIA strips |
| CAL | Калибровочные пробы / Calibrator set |
| CONTROL | Контрольная сыворотка / Control sera |
| CONJ HRP | Конъюгат / Conjugate |
| SUBS TMB | Раствор субстрата тетраметилбензидина (ТМБ)/ Substrate solution |
| BUF WASH 26X | Концентрат отмывочного раствора / Washing solution concentrate |
| STOP | Стоп-реагент / Stop solution |
| DIL | ИФА-Буфер / EIA buffer |