Allergen-related IgG4 Antibody Screen ELISA Kit (20 Food Antigens)

Enzyme Immunoassay for the quantitative determination of Allergen-specific IgG4 antibodies in human serum and plasma

Catalog number: ARG80821

For research use only. Not for use in diagnostic procedures.

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PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. 20 allergens and 4X a reference allergen (egg white, f01) are bound to the wells in microtiter strips coded by 3 colors. Diluted serum or ready to use standards are added into wells and the IgG4 existed in samples or standards will bind to the allergens in the wells. After 1 hour incubation at 37°C, the wells are washed with diluted washing solution to remove unbound material. Anti-human-IgG4-AP conjugate is added and the plate is incubated for 30min at 37°C. Following another washing step, a substrate solution (PNPP) is pipetted the plate is incubated for 60min at 37°C and a yellow color is developed in the wells. The color development is inhibited by the addition of a stop solution, and the color is measured at 405 nm. The concentration of IgG4 antibodies is directly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Microtiter plate	12 strips X 8 wells (for 4 patients). Detailed information of antigen coating is described on page5 (Reagent Preparation)	4°C
Standards (0.35, 0.70, 3.5, 17.5 U/ml IgG4 antibodies against f01)	4 X 0.5 ml (ready to use)	4°C
Sample dilution buffer	40 ml (ready to use)	4°C

Mouse anti human IgG4-AP-conjugate	15 ml (ready to use)	4°C
10X Wash buffer	60 ml	4°C
PNPP substrate	15 ml (ready to use)	4°C (Protect from light)
STOP solution	15 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 405nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the Antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer and Sample diluent buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

In general, serum or plasma (EDTA, heparin) can be used for the determination. From the aseptic with venipuncture collected blood the serum is separated after coagulation with centrifugation. The serum or plasma samples can be stored up to 2 days at 2-8°C, for longer storage the samples should be frozen at –20°C. Avoid repeated freezing and thawing. Lipaemic, haemolytic or contaminated samples can lead to incorrect results. For the performance of the assay the samples (not the standards) have to be diluted 1:101 with the ready to use sample diluent (e.g. 25µl serum + 2.5ml sample diluent). For 20 tests/screen only 25µl serum are needed.

REAGENT PREPARATION

- Microtiter Strips: The kit includes enough material for 96 determinations for 4 patient samples. For each patient, 3 color-coded strips (green, yellow, red) are provided. Hence the kit provides 4 green strips, 4 yellow strips and 4 red strips altogether.

  3 strips with 8 wells coated with 20 different allergens should be assayed together for each patient. In the green strips, 4 different allergens and 4X reference allergen in different concentrations are coated on the wells. In the yellow and red strips, 16 different allergens are coated on the wells. The sequence of each allergen coated on wells are as follow:

  Green: 4X reference allergen (egg white, f01), egg white, cow milk, codfish, wheaten flour.

Yellow: Rye flour, barley flour, orange, banana, kiwi, strawberry, celeriac, soy.

Red: Carrot, tomato, hazelnut, peanut, curry, pepper, sesame, pork.

- 1X Wash buffer: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use.

1. For each sample, prepare three microtiter strips (order: green, yellow, red)
2. Remove microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
3. Add 100 µl of ready-to-use standards into first four wells of green stripe.
4. Add 100 µl of 1:101 diluted samples into remaining wells.
5. Incubate for 60 minutes at 37°C (alternatively 120 min at RT).
6. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X wash buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
7. Add 100 µl of Mouse anti human IgG4-AP-conjugate into each well. Incubate for 30 minutes at 37°C (alternatively overnight at RT).
8. Aspirate and wash well as step 6.
9. Add 100 µl of PNPP substrate to each well. Incubate for 60 minutes at 37°C

in dark. (alternatively 90min at RT).

10. Add 100 µl of Stop Solution to each well.
11. Read the OD with a microplate reader at 405 nm immediately.

CALCULATION OF RESULTS

The evaluation can be done in units per ml (U/ml) or in classes.

Example:	Class	OD value
Standard 0.35 U/ml	1	0.145
Standard 0.70 U/ml	2	0.250
Standard 3.50 U/ml	3	0.620
Standard 17.5 U/ml	4	1.715

The values above are only an example found under specific temperature conditions and surrounding. These are no reference values that must be found like this in other laboratories.

Quantitative Evaluation: The ready to use standards of the IgG4 Screen Kit are equilibrated for units per ml (U/ml). This allows an exact and reproducible quantitative evaluation. For the evaluation the extinctions of the standards are graphically plotted vs. the given concentrations. From the resulting calibration curve the concentrations of the patient samples can be determined by finding the respective extinction on the calibration curve and reading the correlating concentration. Automatic evaluation programs can also be used.

QUALITY ASSURANCE

Analytical Sensitivity

Egg-white: 0.22 U/ml; Cow milk: 0.17 U/ml; Tomato: 0.16 U/ml

Intra-assay precision

Egg-white: 7.7%; Cow milk: 8.0%; Tomato: 8.7%

Inter-assay precision

Egg-white: 6.6-10.9%; Cow milk: 8.4-13%; Tomato: 4.6-7.4%

Linearity

Egg-white: 82-114%; Cow milk: 73-100%; Tomato: 102-120%

Recovery

Egg-white	90-107%
Cow milk	89-103%
Tomato	87-97%

Specificity

No cross-reactivity to IgE up to 100 IU/ml