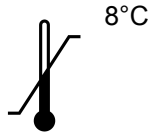




2°C



Please read instructions for use carefully before starting the assay

IgG4 – ELISA

Enzyme Immuno Assay for the quantitative determination of specific IgG4 in human Serum or Plasma

REF

10104PG



96 Determinations

BACKGROUND

Immunoglobulins (Ig) of the IgG4-subclass play an important role in the humoral immune reaction during immunotherapy e.g. for insect venoms (bee and wasp). Increase of the specific IgG4 concentration indicates a positive response to the immunotherapy. Specific IgG4 has been reported to compete with specific IgE for binding to the corresponding antigen (allergen). Therefore specific IgG4 is thought to reduce the capability of degranulation of the mast cells and the basophile granulocytes.

INTENDED USE

The IgG4 - ELISA Test is intended for the determination of specific IgG4 antibodies in human serum or plasma and thus designed to monitor specific IgG4-titres during hyposensitisation treatment.

PRINCIPLES

The test system works on the basis of an Enzyme Linked Immuno Sorbent Assay (ELISA) and detects antigen- (allergen-) specific IgG4-antibodies in serum or plasma from patients.

Diluted human serum or plasma is incubated in antigen (allergen) coated strips. Specific IgG4-antibodies react with their corresponding allergen. Unbound antibodies are removed by washing. The allergen-specific bound antibodies are recognised by a second anti-human IgG4-antibody conjugated to horseradish peroxidase (HRP).

The presence of bound IgG4/anti-IgG4/HRP-complexes is enzymatically detected by incubation with the substrate TMB solution resulting in the development of a blue colour. After stopping the test with acid the colour changes into yellow. The optical density of the yellow colour is measured spectrophotometrically at 450 nm (reference wavelength 620 nm). Within the measuring range the concentration of allergen specific IgG4-antibody is directly proportional to the colour intensity.

Calibrators with defined concentrations of IgG4 are assayed simultaneously with the patient samples to generate a calibration curve. Unknown IgG4 concentrations of the test samples are calculated from this curve.

REAGENTS

Component	Symbols	10104PG
Anti-IgG4-Enzyme-Conjugate	CONJ HRP G₄	1 x 10 mL
Concentrated Washing Buffer B	WASHBUF B 25x	1 x 30 mL
TMB Substrate	SUB TMB	1 x 12 mL
Stop Solution (0.5 M H ₂ SO ₄)	STOP H₂SO₄	1 x 6 mL
Dilution Buffer	DILBUF B	1 x 100 mL

MATERIAL NEEDED, BUT NOT INCLUDED IN THE KIT

1. Referencekit	REF	12004 PG
Reference wells	REFWELL	6 x 8 wells
Calibrators	CAL	4 x 1 mL (100, 250, 500, 2500 U/mL) WHO 67/97 calibrated
Control high	CONTROL H	1 x 0.5 mL
Control low	CONTROL L	1 x 0.5 mL

2. Antigen-coated strips	REF	13-code-G
--------------------------	------------	-----------

OTHER

Pipettes: 10-100 µL, 200-1000 µL, Multipipette, pipette tips, tubes for dilution of the specimens, microplate-reader, incubator, covering foil, lab watch.
Optionally: microplate-washer

SPECIMEN COLLECTION & PREPARATION

Serum or plasma can be used with this test. No additives or preservatives are necessary to maintain the integrity of the specimen.

At 2-8°C specimens are stable for one week. By lengthy storage specimens need to get frozen. As in the case of most proteinous material, repeated freezing and thawing should be avoided. The use of haemolysed and lipemic specimens is not recommended. Specimens should be allowed to come to room temperature (RT) and mixed thoroughly by gentle inversion before assaying. The specimens should be diluted 1:101 (10µL Serum + 1 mL dilution buffer)

PREPARATION OF REAGENTS

Before starting the assay let all samples and reagents come to RT.

Dilution Buffer: ready to use

Conjugate: ready to use

Calibrators: ready to use

Controls: ready to use

TMB Substrate: ready to use

Stop Solution ready to use

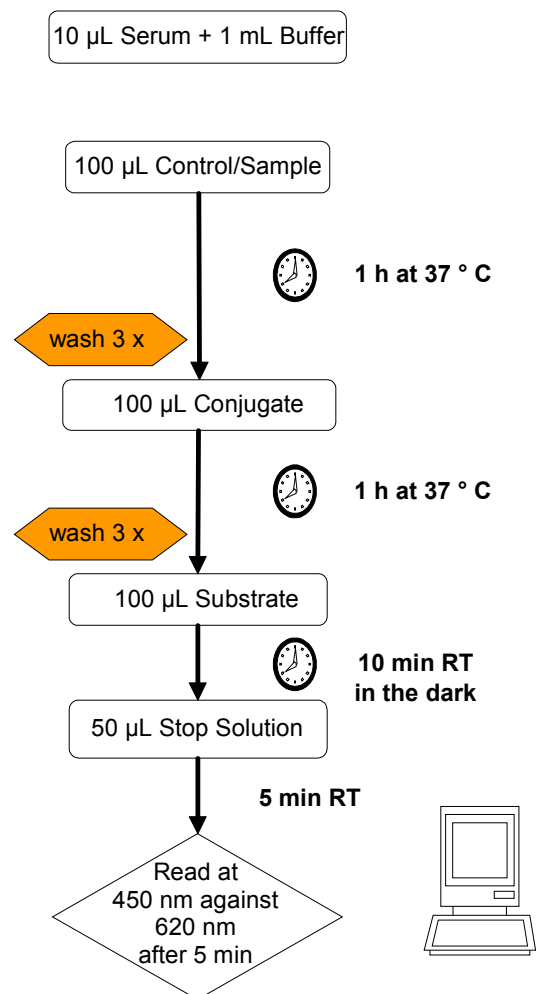
Wash Solution: Dilute 30 mL Washing Buffer Concentrate with distilled water (end volume 750 mL). The resulting Washing Buffer is stable for one week at RT.

ASSAY-PROCEDURE

1. Prepare a protocol for the assay run. It is recommended to test the Calibrators and Controls in duplicate determination.
2. Dilute patient sera **1:101** with dilution buffer (10 µL serum + 1 mL Dilution Buffer).
3. Place a reference strip and the required amount of antigen coated test strips in an appropriate frame. Reseal remaining wells with a desiccant in the aluminium bag.
4. Pipette 100 µL of the Calibrators and Controls and 100 µL of the diluted patient samples into the correspondent wells (see plate matrix).
5. Cover the strips and plate(s) with a foil to prevent evaporation and incubate for 1 h at 37 °C.
6. After the incubation time aspirate solution from all wells and wash each well 3 x with 500 µL wash solution/well. The washing procedure can be performed manually or using a validated microplatewasher. Remove residual liquid by dunking the plate on a tissue.
7. Pipette 100 µL Conjugate into each well and cover the strips with foil. Incubate 1 h at 37 °C.

8. Perform the washing procedure as described in step 6.
9. Pipette 100 µL TMB Substrate into each well and cover the strips with a foil and incubate 10 min in the dark at RT.
10. Pipette 50 µL Stop Solution in chronological order of the Substrate Solution into each well and mix gently.
It is recommended to mix the solutions in the wells by tipping against the frame.
11. Wait 5 min for homogenisation of substrate. Read the optical density using an ELISA Reader at 450 nm (Reference wavelength 620 nm) immediately.

TEST SCHEME IgG4 – ELISA



DR . FOOKE

Laboratorien GmbH

Habichtweg 16

4 1 4 6 8 Neuss

E-mail: information@fooke-labs.de

Internet: www.fooke-labs.de

Tel.: 0049-2131-2984-0

Fax: 0049-2131-2984-184

CALCULATION OF RESULTS

It is recommended to use validated software for the calculation of the results. For manual calculation, the mean OD [Δ 450 nm – 620 nm] values are calculated from the Calibrators and Controls. Generate a graph from the mean OD values of the four Calibrators on half logarithmic paper (Abscissa: log U IgG4/mL; Ordinate: linear OD Δ 450 nm – 620 nm) to create a standard curve. The IgG4 concentration of the patient sample is determined on the basis of this standard curve. The OD is mapped on the Ordinate and the result in U/mL can be read out on the Abscissa. The standard curve and the controls should be in the acceptance range given in the Quality-Control-Certificate delivered with the kit. Otherwise, the test conditions should be verified and the test should probably be repeated.

MEASURING RANGE

IgG4-concentrations between 100 U/mL and 2500 U/mL can be determined.

Sera with IgG4-concentrations above 2500 U/mL should be used in higher dilutions and retested for exact determination of IgG4-content.

INTERPRETATION OF RESULTS:

spez. IgG4. (U/mL)	Interpretation
< 100	negative
100-250	borderline
>250	positive

RESULTS TO BE EXPECTED

The clinical relevance of a positive test report varies significantly between individual antigens. Therefore, it is recommended, that expected values for given populations should be determined by each laboratory over a period of time and in a statistically significant number of assays before clinical significance is attached to the results of the assay. The values given above can be used as a guideline for the own results.

PRECISION

Variability and Reproducibility

1. Intra-Assay (n=10)

SPECIMEN	U/mL	CV (%)
1	204.8	7.55
2	140.4	14.88
3	215.4	6.99

2. Inter-Assay (n=20)

SPECIMEN	U/mL	CV (%)
1	212.3	10.15
2	139.9	17.7
3	230.4	9.08

Example of an calibrator curve:

CALIBRATOR CONCENTRATION	Mean OD 450 nm	Range OD 450 nm
100 U/mL	0.182	0.150 ± 0.10
250 U/mL	0.345	0.250 ± 0.15
500 U/mL	0.595	0.600 ± 0.20
2500 U/mL	2.000	2.000 ± 0.60

LITERATURE:

1. Aalberse R.C. et al.: **Allergen-specific IgG4 in atopic disease.** *Allergy* 1993, **48**:559-569.
2. Garcia et al.: **Modifications in IgG subclass in the course of immunotherapy with grass pollen.** *J Invest Allergol Clin Immunol* 1993, **3**:19-25.
3. Harris et al.: **The development of specific IgG4 after immunotherapy with standardized extracts.** *N Engl Reg Allergy Proc* 1987, **8**:429-435.
4. Hedlin et al.: **Long-term follow up of patients treated with a three-year course of cat or dog immunotherapy.** *J Allergy Clin Immunol.* 1995, **96**:879-885.
5. Van der Zee & Aalbers: **IgG4 and hyposensitization.** *N Engl Reg Allergy Proc* 1987, **8**:389-391.

PRECAUTIONS FOR USERS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of *in-vitro* diagnostic medical devices is intended to secure suitability, performance and safety of the product by the manufacturer. Therefore the test procedure, information, precautions and warnings stated in the instructions for use have to be followed strictly. The kit has only to be used as described on page 1 (intended use).
- The test must be performed according to this instruction, which contains all necessary information, precautions and warnings. The use of the test kit with analyzers and similar equipment has to be validated. Any change in design, composition of the test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes resulting in false results and other incidents. The manufacturer is not liable for any results obtained by visual analysis of patient samples.
- The kit is intended for use by trained and qualified professionals carrying out research or diagnostic activities only. Pregnant women should not perform the test.
- Laboratory equipment has to be maintained according to the manufacturer's instructions and must be tested for its correct function before use.
- For *in-vitro* diagnostic use only. Use only once. Do not use components exceeding the expiry date. Do not combine reagents of other suppliers or kit components of different lots (unless specified on page 1) with this kit.
- Do not use kit components when the package of the component is damaged. Please check all solutions prior to use for microbiological contamination. Cap vials tightly immediately after use to avoid evaporation and microbiological contamination. Do not interchange screw caps of the reagent vials.
- The kit was evaluated for use at the temperatures specified in the Testing scheme (see page 2). Higher or lower temperatures may result in values not meeting the quality control ranges.
- The washing procedure is absolutely important. Improper washing will cause erroneous results. It is recommended to use a multichannel pipette and an automated washer.
- To avoid cross-contamination and false-positive results it is recommended to perform all pipetting steps properly. Use only clean pipette tips, dispensers and lab ware.
- Test components based on human serum were tested using a CE marked method for the presence of antibodies against HIV 1 / HIV 2, Anti-HBc, and Anti-HCV as well as for hepatitis antigen HBsAg and were found to be negative. Nevertheless, material based on human serum should be handled as potentially infectious (BIOHAZARD).
- Some kit components may contain bovine serum albumin, of which according to the manufacturer no infectious potential is known. Due to the eventual occurrence of undetectable infectious agents we recommend to handle any product of animal origin as potentially infectious.
- The following safety rules should be followed with all reagents:
 - Do not get in eyes, on skin, or on clothing (P262). Do not breathe spray (P260). Pipetting should never be done by mouth, but with suitable pipetting devices.
 - IF SWALLOWED: rinse mouth. Do NOT induce vomiting (P301/330/331)
 - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower (P303/361/353).
 - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing (P303/340).
 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305/351/338)
 - Don't eat, drink or smoke while performing the test. Keep away from food, feed and beverage.
 - Wear protective gloves/protective clothing/eye protection (P280). Wash hands thoroughly after handling (P264) and care for your skin.
 - Material safety data sheet is available on request.
- Stop Solution causes severe skin burns and eye damage (H314).
- TMB in high concentrations may be potentially mutagenic. Due to the low concentration of TMB in this substrate solution a mutagenic effect can be ruled out, if it is properly used.
- The preservatives (Bronidox, Thimerosal, Azid) are toxic to aquatic life, but their concentration is not hazardous to environment anymore. On disposal, flush large volumes of reagents with plenty of water. Thimerosal (WashBuf B) may cause damage to organs through prolonged or repeated exposure (H373)
- Waste containing serum must be collected in separate containers containing an appropriate disinfectant in sufficient concentration. This material has to be treated according to national biohazard and safety guidelines or regulations.
- We refer to the national regulations of medical devices regarding *in-vitro* diagnostic test kits.



DR. FOOKE

Laboratorien GmbH Tel.: 0049-2131-2984-0
 Habichtweg 16 Fax: 0049-2131-2984-184
 4 1 4 6 8 Neuss
 E-mail: information@fooke-labs.de
 Internet: www.fooke-labs.de

Lot-Number	European conformity	For <i>in-vitro</i> diagnostic use	Temperature Limit	Use before	Catalogue Number	Consult instructions for use	Refer accompanying documents	Do not use when package is damaged	Do not Re-use	Sufficient for <n> tests	Manufactured by	Biohazard