CE IVD 2°C 8°C

Please read instructions for use carefully before starting the assay

Specific IgG - ELISA

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

REF 10100PG Σ 96 Determinations

BACKGROUND

Antibodies, also known as immunoglobulins (Ig), play an important role in the humoral immune response to extrinsic, infectious agents. Furthermore Ig and in particular Ig of the IgG type can be generated to normally harmless antigens, e.g. inhalation antigens or food antigens. A typical disease represents the exogenic allergic alveolities (EAA), which can be caused by occupational exposition of substances in the air. Involved are normally mouldy- and bacterial components but also excrements, flours or chemical components which are inhaled as dusts or aerosols during work. Farmer´s lung is caused by *Thermophile Actinomycetes* in mouldy hay. Farmer´s lung, Malt- and Paper worker´s lung as well as Pigeon Breeder´s disease are well known to represent such diseases. Also fish flour, saw dust, dust from furs and in addition chemical components can cause Alveolitis.

INTENDED USE

The Specific IgG - ELISA is intended for the quantitative determination of specific IgG in human serum and plasma against inhalation antigens and help to diagnose so called Type III reactions of the lung (e.g. Farmer´s lung, Malt and Paper worker´s lung and Bird fancier´s disease).

PRINCIPLES

This sIgG test is based on ELISA (**E**nzyme **L**inked **I**mmuno **S**orbent **A**ssay) technology. It detects specific IgG of patients with Type III allergies (exogenic allergic alveolities). Diluted serum or plasma samples of patients are incubated in antigen coated microwell strips. During incubation specific IgG binds to antigens on the surface of the microwell cavity. After application of anti-human-IgG-alkaline-phosphatase (AP) conjugate a complex of antigen/IgG/anti-IgG-AP will form. After application of p-nitrophenylen-phosphate (pNPP) solution the amount of antibody-enzyme complex is detected. After addition of sodium hydroxide (NaOH) the extinction of the coloured reaction product which is proportional to the amount of specific IgG of the sample, can be measured at 405 nm (reference wave length 620 nm).

Calibrators of know IgG concentrations form a calibration curve in which the IgG concentrations of the samples can be determined.

REAGENTS

Component	**Symbols**	**10100PG**
Anti-IgG-Enzyme Conjugate	CONJ AP G	1 x 10 mL
Washing Buffer Concentrate C	WASHBUF C 50x	1 x 30 mL
Dilution Buffer	DILBUF C	1 x 100 mL
Substrate Buffer	SUBBUF	1 x 25 mL
Substrate Tablets (pNPP)	SUB PNPP	5 pieces
Stop Solution (1 N NaOH)	STOP NAOH	1 x 10 mL

MATERIAL NEEDED, BUT NOT INCLUDED IN THE KIT

1. Reference unit	**Symbols**	**12001PG**
Reference wells	REFWELL	6 x 8 wells
Calibrators	CAL	5 x 1.0 mL (**0.25; 0.83; 2.5; 8.3; 25 U/mL**)
Control high	CONTROL H	1 x 1.0 mL
Control low	CONTROL L	1 x 1.0 mL

2. Antigen-coated plates	MICROWELL	**13-code-G**

OTHERS

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

SPECIMEN COLLECTION & PREPARATION.

Either Serum or Plasma can be used in this test. No additives or preservatives are necessary to maintain the integrity of the specimen.
Specimens should be stored at 2-8°C and assayed within 48 hours after collection. If the assay cannot be performed within 48 hours or if the specimen has to be shipped, cap the specimen and keep it frozen. Repeated freezing and thawing should be avoided. Frozen specimens should be thawed at room temperature (RT, 20-25°C) and mixed thoroughly by gentle inversion before assaying. Samples should be tested undiluted. The use of haemolysed or lipemic specimens is not recommended.

PREPARATION OF REAGENTS

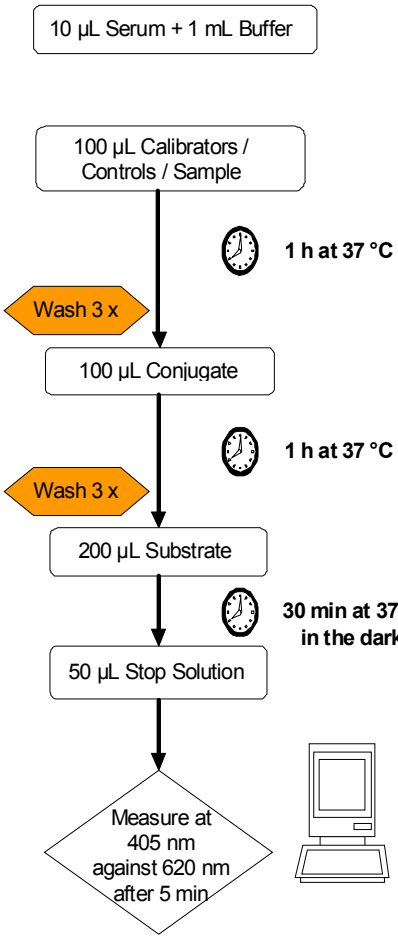
Microtiterstrips which are not required have to be re-sealed properly in the provided foil bag containing a desiccant.
Allow all reagents to come to RT before use.

Conjugate: ready to use
Calibrators and Controls: ready to use
Dilution Buffer: ready to use
Stop Solution: ready to use
Substrate Solution:
For 20 determinations dissolve one Substrate Tablet in 5 mL Substrate Buffer about one hour before use and store in the dark.
Wash Solution:
The concentrated Washing Buffer has to be diluted 1:50 in aqua bidest. (Example: For 2 strips 10 mL of Washing Buffer is required. Therefore 200 µL concentrated Washing Buffer have to be diluted to a final volume of 10 mL with aqua bidest.). 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 samples **1:101** with Dilution Buffer (10 µL serum + 1 mL Dilution Buffer).
3. Place the required coated wells into a frame and reseal the aluminium bag with the remaining strips and desiccant properly.
4. Pipette exactly 100 µL calibrators, controls and patient samples into their respective wells.
5. Cover plate and incubate for one hour at 37 °C.
6. Wash the plate manually or with an appropriate ELISA plate washer 3 times each with 300 µL Washing Buffer per well. Remove residual liquid by dunking the microplate onto a paper towel.
7. Pipette exactly 100 µL anti-IgG conjugate in each well and cover the plate and incubate for one hour at 37 °C.
8. Repeat washing as described in step 6.
9. Pipette 200 µL Substrate Solution (pNPP in Substrate Buffer) to each well, cover and incubate for 30 min at 37 °C in the dark.
10. Pipette 50 µL of Stop Solution in the same order as the substrate to each well.
11. Incubate for 5 min at RT to let the solution homogenize.
12. Read OD at 405 nm (reference wave length 620 nm) using an appropriate microplate reader which is maintained regularly.

TEST SCHEME Specific IgG - ELISA



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

CALCULATION OF RESULTS

It is recommended to use validated software for the calculation of the results. For manual calculation, the mean OD [Δ 405 nm – 620 nm] values are calculated from the Calibrators and Controls. Generate a graph from the mean OD values of the five Calibrators on half logarithmic paper (Abscissa: log U IgG/mL; Ordinate: linear OD Δ 405 nm – 620 nm) to create a standard curve. The sIgG 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-Certificates delivered with the kit. Otherwise, the test conditions should be verified and the test should be repeated probably.

MEASURING RANGE

This ELISA detects IgG concentrations between 0.25 U/mL and 25 U/mL. Specimens with higher IgG concentrations should be diluted and retested to determine the exact IgG content.

Interpretation of results:

spec. IgG (U/mL)	Interpretation
< 1.0	negative
1.0 – 2.0	indefinite
> 2.0 – 3.5	positive
> 3.5	strong 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.

ALLERGEN WELLS WITH CONTROL WELLS (HSA)

Low molecular substances (Haptens) e.g. Penicillin and Isocyanates, are coupled to the microtiterstrips by a protein (Human Serum Albumin / HSA). In rare cases patient samples can contain HSA specific IgG. Therefore reaction against HSA itself has to be tested for each patient sample by running the HSA-Control well test and comparing the results to the Antigen-HSA-Conjugate.

Recommended interpretation:

The sIgG concentration against the HSA conjugate is measured in parallel to sIgG to HSA. The sIgG concentration to HSA has to be subtracted from the concentration obtained from the respective HSA conjugate.

Alternative interpretation:

The result for the Antigen-HSA-Conjugate is calculated by multiplying the OD-Value of the HSA Control Disc by the factor 2.

Cut off = OD (HSA control disc) X 2

OD Antigen-HSA-Conjugate > Cut off: positive result.

PRECISION

Variability and Reproducibility

1. ***Intra***-Assay

Specimen	U/mL	CV (%)
1 (n=10)	178	5.8
2 (n=10)	53	6.6
3 (n=10)	237	7.0

2. ***Inter***-Assay

Specimen	U/mL	CV (%)
1 (n=20)	161	13.5
2 (n=20)	58	10.5
3 (n=20)	239	7.7

EXEMPLARY CALIBRATOR CURVE

Calibrator-Concentraitons (U/mL)	Mean OD 405 nm	Range OD 405 nm
0.25	0.173	0.200 ± 0.100
0.83	0.298	0.300 ± 0.100
2.5	0.661	0.750 ± 0.150
8.3	1.774	1.800 ± 0.300
25	2.820	3.000 ± 0.500

LITERATUR:

1. Garcia et al: **Modifications in IgG subclass in the course of immunotherapy with grass pollen.** *J Invest Allergol Clin Immunol* 1993, **3:**19-25.

2. 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:**79-85.

3. Moss RB: **IgG Subclass Antibody Markers in Grass pollen Immunotherapy.** *N Eng Reg Allergy Proc* 1987, **8**:409-415.

4. Nakagawa T: **IgG Subclass Antibodies in Response to House Dust Mite Immunotherapy**. *N Eng Reg Allergy Proc* 1987, **8:**423-428.

PRECAUTIONS FOR USERS

1. 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).
2. 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.
3. 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.
4. Laboratory equipment has to be maintained according to the manufacturer's instructions and must be tested for its correct function before use.
5. 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.
6. 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.
7. 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.
8. The washing procedure is absolutely important. Improper washing will cause erroneous results. It is recommended to use a multichannel pipette and an automated washer.
9. 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.
10. 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).
11. 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.
12. 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.
13. Stop Solution and SubBuf cause severe skin burns and eye damage (H314).
14. 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.
15. p-NPP is harmful if swallowed (H302). Diethanolamin (SubBuf) may cause damage to organs through prolonged or repeated exposure (H373). Get medical advice/attention if you feel unwell (P314).
16. The preservatives (Bronidox) 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.
17. 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.
18. We refer to the national regulations of medical devices regarding *in-vitro* diagnostic test kits.

D R . F O O K E
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	CE	IVD	2 °C 8 °C		REF					Σ		
Lot- Number	European conformity	For *in-vitro* 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