Gliadin IgA ELISA Kit

Catalog Number KA1286

96 assays

Version: 05

Intended for research use only

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Introduction

Intended Use

The Gliadin IgA ELISA Kit has been designed for the detection and the quantitative determination of specific IgA antibodies against Gliadin in serum and plasma. Further applications in other body fluids are possible and can be requested from the technical service of Abnova. This assay is intended for research use only.

Background

Gliadin is the main component of gluten, which occurs in wheat and other domestic grain types like rye, barley and oats, and may lead to severe diseases of the intestinal mucosa in sensitive children and adults. Celiac disease, a gluten-induced enteropathy, appears rather frequently (1 case on 300 births) and is a typical example of a non-IgE mediated food allergy. Genetically, histocompatibility antigens on the chromosome 6 are responsible for the disease. Celiac disease manifests itself practically as a constant reaction against gliadin. By the toxic effect of gluten in the intestinal tract, antibodies, cytokines and lymphocytes are released, which lead to internal lesions and inflammations. Further, the microvilli of the intestine are almost completely reduced, so that the inner intestinal surface becomes flat. The resulting malabsorption leads to a deficit of above all trace elements and vitamins. Loss of weight, diarrhea, flatulence and abdominal pain are observed as symptoms. An invasive diagnostic possibility represents the biopsy of the intestinal mucosa. In addition serological methods for the determination of IgG and IgA antibodies against gliadin, reticulin and endomysium in the patient serum are increasingly used as a screening method. For children with a gluten-sensitive enteropathy, the incidence was calculated to 90-100%, for adults with celiac disease 75-90% and for dermatitis herpetiformis 40-50%. Elevated levels of IgA anti-gliadin demonstrate an active process and are in close correlation with a villous atrophy in children. The ELISA antibody determination is also well suited for the monitoring of persons after a gluten-free diet.

Principle of the Assay

The Gliadin IgA ELISA Kit is based on the principle of the enzyme immunoassay (EIA). Gliadin antigen is bound on the surface of the microtiter strips. Diluted serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgA antibodies of the serum and the immobilized Gliadin antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgA peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgA antibodies is directly proportional to the intensity of the color.

General Information

Materials Supplied

List of component

Component	Amount.
Microtiter strips: 12 strips with 8 breakable wells each, coated with a Gliadin antigen (purified gluten antigen from wheat). Ready-to-use.	96 (8 x 12) wells
Calibrator A (Negative Control): Protein solution diluted with PBS, contains no IgA antibodies against Gliadin. Addition of 0.01% methylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.	2 mL
Calibrator B (Cut-Off Standard): Human serum diluted with PBS, contains a low concentration of IgA antibodies against Gliadin. Addition of 0.01% methylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.	2 mL
Calibrator C (Weak Positive Control): Human serum diluted with PBS, contains a medium concentration of IgA antibodies against Gliadin. Addition of 0.01% methylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.	2 mL
Calibrator D (Positive Control): Human serum diluted with PBS, contains a high concentration of IgA antibodies against Gliadin. Addition of 0.01% methylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.	2 mL
Enzyme Conjugate: Anti-human-IgA-HRP (rabbit), in protein-containing buffer solution. Ready-to-use.	15 mL
Substrate Solution: TMB (tetramethylbenzidine). Ready-to-use.	15 mL
Stop Solution: 1 N acidic solution. Ready-to-use.	15 mL
Sample Diluent: PBS/BSA buffer. Addition of 0.095% sodium azide. Ready-to-use.	60 mL
Washing Buffer (10x): PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.	60 mL

Storage Instruction

Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. After the first opening the kit should be used within 3 months, the diluted wash buffer can be kept for 4 weeks at 2-8°C.

Materials Required but Not Supplied

- ✓ 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- ✓ Microtiter Plate Reader (450 nm)
- ✓ Microtiter Plate Washer
- ✓ Reagent tubes for the serum dilution
- ✓ Bidistilled water
- ✓ Re-usable black lid for covering
- ✓ Plastic bag

Precautions for Use

- Precautions

1. Only for research use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
2. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
3. Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5% and have to be disposed of properly.
4. All reagents have to be brought to room temperature (18 to 25°C) before performing the test.
5. Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
6. It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
7. When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
8. In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
9. No reagents from different kit lots have to be used, they should not be mixed among one another.
10. All reagents have to be used within the expiry period.
11. In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
12. The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

Assay Protocol

Reagent Preparation

Washing Solution:
Dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

Sample Preparation

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20°C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.
For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

Assay Procedure

- ✓ Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- ✓ All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- ✓ Standards and samples should be assayed in duplicates.
- ✓ A standard curve should be established with each assay.
- ✓ Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

1. Prepare a sufficient amount of microtiter wells for the standards, controls and samples in duplicate as well as for a substrate blank.
2. Pipet 100 µL each of the diluted (1:101) samples and the ready-to-use standards and controls respectively into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the re-usable plate cover and incubate at room temperature for 60 minutes.
4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
5. Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6. Cover plate with the re-usable plate cover and incubate at room temperature for 30 minutes.
7. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This

procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

8. Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.
9. Cover plate with the re-usable plate cover and incubate at room temperature for 20 minutes in the dark (e.g. drawer).
10. To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

Data Analysis

Calculation of Results

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%.

Example:

	OD Value	Corrected OD	Mean OD Value
Substrate Blank	0.020		
Negative Control	0.058 / 0.061	0.038 / 0.041	0.040
Cut-off Standard	0.546 / 0.538	0.526 / 0.518	0.522
Weak Positive Control	0.918 / 0.904	0.898 / 0.884	0.891
Positive Control	1.522 / 1.418	1.502 / 1.398	1.450

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently no reference value which have to be found in other laboratories in the same way.

- ✓ Qualitative Evaluation

  The calculated absorptions for the sample sera, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result. For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of +/-20% around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same donor, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run. The positive control must show at least the double absorption compared with the cut-off standard.

- ✓ Quantitative Evaluation

  The ready-to-use standards and controls of the Gliadin antibody kit are defined and expressed in arbitrary units (U/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given donor follow-up controls become possible. The values of controls and standards in units are printed on the QC data sheet. For a quantitative evaluation the absorptions of the standards and controls are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each sample can then extracted in relation to their absorptions. It is also possible to use automatic computer programs.

Performance Characteristics

Gliadin ELISA	IgA
Intra-Assay-Precision	9.6%
Inter-Assay- Precision	10.1%
Inter-Lot-Precision	4.7 – 10.1%
Analytical Sensitivity	1.05 U/mL
Recovery	70 - 119 %
Linearity	73 - 126 %
Cross-Reactivity	No cross-reactivity to TG, TPO, dsDNA and Transglutaminase
Interferences	No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL
Specificity	100%
Sensitivity	100%

Resources

References

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Plate Layout

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