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Instruction Manual

Gliadin IgA ELISA

Enzyme immunoassay
for the detection and quantitative determination
of human IgA antibodies against **Gliadin**
in serum and plasma

CE

Cat. No.:	DEGLI02
Storage:	2-8°C
Regulatory:	For in-vitro diagnostic use only

Contents

1. Intended Use

The DEMEDITEC Gliadin IgA Antibody ELISA Test Kit has been designed for the 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 DEMEDITEC.

Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

2. General Information

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 patients after a gluten-free diet.

3. Principle of the Test

The DEMEDITEC Gliadin IgA antibody test kit is based on the principle of the enzyme immunoassay (EIA). Gliadin antigen is bound on the surface of the microtiter strips. Diluted patient 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.

4. Limitations, Precautions and General Comments

- Only for in-vitro 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.
- 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.
- 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.
- All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- 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.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- No reagents from different kit lots have to be used, they should not be mixed among one another.
- All reagents have to be used within the expiry period.
- 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.
- 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.

5. Reagents Provided

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. The opened kit should be used within three months.

Components	Volume / Qty.
Gliadin antigen coated microtiter strips	12
Calibrator A (Negative Control)	2 mL
Calibrator B (Cut-Off Standard)	2 mL
Calibrator C (Weak Positive Control)	2 mL
Calibrator D (Positive Control)	2 mL
Enzyme Conjugate	15 mL
Substrate	15 mL
Stop Solution	15 mL
Sample Diluent	60 mL
Washing Buffer (10×)	60 mL
Plastic bag	1

5.1. Microtiter Strips
12 strips with 8 breakable wells each, coated with a Gliadin antigen (purified gluten antigen from wheat). Ready-to-use.

5.2. Calibrator A (Negative Control)
2 mL, protein solution diluted with PBS, contains no IgA antibodies against Gliadin. Addition of 0.01 % methyl-isothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.3. Calibrator B (Cut-Off Standard)
2 mL 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.

5.4. Calibrator C (Weak Positive Control)
2 mL, 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.

5.5. Calibrator D (Positive Control)
2 mL, 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.

5.6. Enzyme Conjugate
15 mL, anti-human-IgA-HRP (rabbit), in protein-containing buffer solution. Ready-to-use.

5.7. Substrate
15 mL, TMB (tetramethylbenzidine). Ready-to-use.

5.8. Stop Solution
15 mL, 1 N acidic solution. Ready-to-use.

5.9. Sample Diluent
60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.

5.10. Washing Buffer
60 mL, 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.

5.11. Plastic Bag
Resealable, for the dry storage of non-used strips.

6. Materials Required but not Provided

- 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
  (Available upon request at Demeditec Diagnostics GmbH)

7. Specimen Collection and Handling

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).

8. Assay Procedure

8.1. Preparation of Reagents

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.

- 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.

8.2. Assay Steps

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.
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.

9. Calculation of Results

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics or Logit-Log.

For the calculation of the standard curve apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

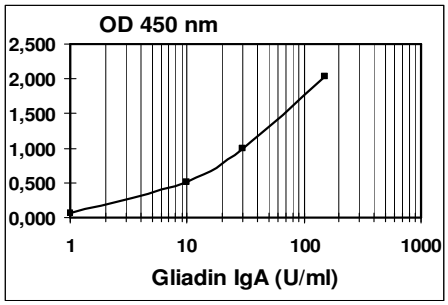
The concentration of the samples can be read from the standards curve.

The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor.

Samples showing concentrations above the highest standard have to be diluted as described in “Assay Procedure” (chapter 8.) and reassayed.

Typical Calibration Curve (Example. Do not use for calibration!)

Standard	U/mL	Mean OD
A (Negative Control)	1	0.067
B (Cut-Off Standard)	10	0.512
C (Weak Positive Control)	30	0.992
D (Positive Control)	150	2.036



10. Interpretation of Results

U/mL	Interpretation
< 8	negative
8 - 12	equivocal
> 12	positive

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

11. Expected Values

In an in-house study apparently healthy subjects showed the following results:

Ig Isotype	n	Interpretation		
		positive	equivocal	negative
IgA	56	1.8 %	0 %	98.2 %

12. Performance / Assay 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
Clinical Specificity	100 %
Clinical Sensitivity	100 %

13. References

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