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User's Manual

Wheat Gliadin IgA ELISA Kit

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Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Gliadin IgA Antibody ELISA Test Kit has been designed for the detection and the quantitative determination of specific IgA antibodies against Gliadin in serum and plasma. This assay is intended for research use only.

General Description

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.

Principles of Testing

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 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.

Reagents And Materials 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.

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

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

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.

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.

Calibrator D (Positive Control): 2 mL, human serum dilute with PBS, contains a high concentration of IgA antibodies against Gliadin. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

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

Substrate: 15 mL, TMB (tetramethylbenzidine). Ready-touse.

Stop Solution: 15 mL, 0.5 M sulfuric acid. Ready-to-use.

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

Washing Buffer: 60 mL, PBS+Tween 20, 10× 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.

Plastic Foils: 2 pieces to cover the microtiter strips during the incubation.

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

Materials Required But Not Supplied

1. 5 µL, 100 µL and 500 µL micro- and multichannel pipets
2. Microtiter Plate Reader (450 nm)
3. Microtiter Plate Washer
4. Reagent tubes for the serum dilution
5. Bidistilled water

Storage

Store kit at 2-8°C. It is stable up to the expiry date stated on the label of the box. Do not use kit beyond its expiry date.

Specimen Collection And 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. 5µL serum+500µL sample diluent).

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.

Assay Procedure

General Remarks:

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

Procedure:

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) sample and the ready-to-use standards or controls respectively into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the enclosed foil 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 enclosed foil 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 enclosed foil and incubate at a 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.

Evaluation

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%.

1. Qualitative Evaluation

The calculated absorptions for the 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 patient, 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.

2. 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 patient follow-up controls become possible. The values for controls and standards in units are printed on the labels of the vials. 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 patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs.

Performance Characteristics

Gliadin ELISA	IgG	IgA	IgM
Intra-Assay-Precision	6.1%	9.6%	8.6%
Inter-Assay-Precision	4.6%	10.1%	9.8%
Inter-Lot-Precision	1.7–4.7%	4.7–10.1%	4.0–13.1%
Analytical Sensitivity	1.11 U/mL	1.05 U/mL	0.94 U/mL
Recovery	73–106%	70–119%	72–126%
Linearity	72–109%	73–126%	79–123%

Specificity

No cross-reactivity to TG, TPO, dsDNA and Transglutaminase.

Interferences

No interferences with bilirubin up to 0.3 mg/ mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL

References

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