Version 12-11/25

User's Manual

Human Anti-Gliadin IgG ELISA Kit

REF **DEIA1827**

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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.

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PRODUCT INFORMATION

Intended Use

This microplate based EIA (enzyme immunoassay) kit is intended for the quantitative determination of human anti-gliadin IgG level in serum.

General Description

Celiac disease or gluten-sensitive enteropathy is characterized by atrophy of the small intestinal villi leading to a so-called flat mucosa occurring in both adults and children. It is caused by a pathological intolerance to gliadin resulting in inflammation and atrophy of the mucosa of the small intestine. Clinical manifestations include malabsorption with symptoms of diarrhea, steatorrhea and nutritional and vitamin deficiencies. Secondary immunologic illnesses, such as atopic dermatitis, dermatitis herpetiformis, alopecia and aphthous ulcers may be the primary presentation. As celiac disease is caused by the uptake of gluten, consequently a gluten-free diet cures the disease completely and thus has to be maintained for lifetime. Renewed consumption of gliadin leads to a recurrence of the disease symptoms. The disease is HLA-associated (>95% of patients have DQ2 encoded by DQA1*0501 and DQB1*0201) and manifests at any age. A high incidence range up to 1:300 was found in European countries and approximately 1:250 in the United States.

Clinical diagnosis of celiac disease is made by small intestinal biopsy and supported by serological markers. Human antibodies against gliadin and tissue Transglutaminase (tTG) are major serological markers. Circulating IgG and IgA antibodies to gliadin are found in the serum of most but not all celiac disease patients. Both IgG and IgA antibodies are detected in sera of patients with gluten-sensitive enteropathy. It was reported that IgA antibodies are less sensitive but more specific markers of the disease and their measurement is useful in following disease activity and monitoring maintenance of a gluten-free diet. IgG antibodies appear to be more sensitive but less specific markers of disease than IgA. It is recommended that both antibodies should be measured due to the high incidence of IgA deficiency among celiac patients, which may mask the disease. Antibody testing is also important in detecting individuals who are at risk for having celiac disease but have no symptoms, in individuals with atypical symptoms or extra-intestinal manifestations of celiac disease and in individuals with presumed celiac disease who fail to respond to a gluten-free diet. Patients with positive antibody tests must undergo small intestine biopsy to confirm the diagnosis and assess the degree of mucosal involvement. Antibodies to gliadin may be the only serological marker in neonates, as anti-tTG and EMA auto-antibodies are not present at this age. Consequently anti-gliadin antibodies are the earliest serological marker for pediatricians when diagnosing celiac disease.

Principles of Testing

This EIA is designed, developed and produced for the quantitative measurement of human anti-gliadin IgG level in test sample. The assay utilizes the microplate based enzyme immunoassay technique by coating highly purified gliadin antigen onto the wall of microtiter well.

Assay calibrators, controls and human serum samples containing anti-gliadin IgG are added to microtiter wells of a microplate that was coated with a highly purified gliadin antigen on its wall. After the first incubation period, the unbound protein matrix is removed in the subsequent washing step. A horseradish peroxidase conjugated rabbit anti-human IgG subclass specific antibody (tracer antibody) is added to each well. After an incubation period an immunocomplex of "gliadin – human anti-gliadin IgG – HRP-conjugated tracer antibody"

is formed if there is human anti-gliadin antibody present in the test sample. The unbound tracer antibody is removed in the subsequent washing step. HRP-conjugated tracer antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to the human IgG on the wall of the microtiter well is directly proportional to the amount of human anti-gliadin antibody level in the sample. A calibrator curve is generated by plotting the absorbance versus the respective human anti-gliadin antibody concentration for each calibrator on point-to-point or 4-parameter fit. The concentration of human anti-gliadin antibody in test samples is determined directly from this calibrator curve.

Reagents And Materials Provided

Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

1. Gliadin Coated Microplate

One microplate with 12 x eight strips (96 wells total) coated with highly purified gliadin antigen. The plate is framed and sealed in a foil Ziploc bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. Anti-hIgG Tracer Antibody

One vial containing 0.6 mL concentrated horseradish peroxidase (HRP)-conjugated anti-human IgG tracer antibody in a stabilized protein matrix. This reagent must be diluted with Tracer Antibody Diluent before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. Tracer Antibody Diluent

One vial containing 12 mL ready-to-use buffer. It should be only used for antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. ELISA Wash Concentrate

One bottle contains 30 mL of 30 fold concentrate. Before use the contents must be diluted with 870 mL of distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate-buffered saline with a non-azidepreservative. The diluted wash solution should be stored at room temperature and is stable until the expiration date on the kit box.

5. ELISA HRP Substrate

One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

6. ELISA Stop Solution

One bottle contains 12 mL of 0.5 M sulfuric acid. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

7. Gliadin IgG Calibrators

Five vials each contain human anti-Gliadin IgG in a liquid bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration for each calibrator. After the first use, the calibrators should be stored at -20°C or below for long term storage.

8. Gliadin IgG Controls

Two vials each contain human anti-Gliadin IgG in a liquid bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration range for each control. After the first use, the calibrators should be stored at -20°C or below for long term storage.

9. Patient Sample Diluent

One bottle contains 60 mL phosphate buffer with protein stabilizers and preservative. The reagent is ready to use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

Materials Required But Not Supplied

1. Precision single channel pipettes capable of delivering 10 µL, 50 µL, 100 µL, and 1000 µL, etc.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 × 75 mm or 13 × 100 glass tubes.
5. Disposable plastic 1000 mL bottle with caps.
6. Aluminum foil.
7. Deionized or distilled water.
8. Plastic microtiter well cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

Specimen Collection And Preparation

Only 10 µL of human serum is required for gliadin IgG measurement in duplicate. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected and must be allowed to clot for minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500xg for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube. Serum samples should be stored at 2 – 8°C up to 48 hours, and at –20°C or below for long term storage until measurement.

Patient Sample Preparation

Patient sample needs to be diluted 1:101 with assay buffer before being measured.

(1) Label a test tube (12×75 mm).

(2) Add 1 mL of assay buffer to each tube. Pipet 10 µL of patient serum sample to the tube.

Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

(2) ELISA Wash Concentrate must be diluted to working solution prior use. Please see **Reagents And Materials Provided** for details.

Assay Procedure

(1) Place a sufficient number of gliadin coated microwell strips in a holder to run gliadin IgG calibrators, controls, and unknown samples in duplicate.

(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	STD 1	STD 5	SAMPLE 2
B	STD 1	STD 5	SAMPLE 2
C	STD 2	C 1	SAMPLE 3
D	STD 2	C 1	SAMPLE 3
E	STD 3	C 2	SAMPLE 4
F	STD 3	C 2	SAMPLE 5
G	STD 4	SAMPLE 1	
H	STD 4	SAMPLE 1	

(3) Add 100 µL of calibrators, controls and diluted patient serum samples into the designated microwell.

(4) Cover the plate with one plate sealer.

(5) Incubate plate at room temperature for 1 hour.

(6) Prepare working anti-hIgG Tracer Antibody Working Solution by 1:21 fold dilution of the tracer antibody with the Tracer Antibody Diluent. For each strip, it is required to mix 1 mL of Tracer Antibody Diluent with 50 µ L of Gliadin IgG Tracer Antibody in a clean test tube.

(7) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µ L to 400

µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.

(8) Add 100 µL of above diluted tracer antibody working solution to each of the wells.

(9) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.

(10) Incubate plate at room temperature for 30 minutes.

(11) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.

(12) Add 100 µL of ELISA HRP Substrate into each of the wells.

(13) Cover the plate with a new plate sealer and also with aluminum foil to avoid exposure to light.

(14) Incubate plate at room temperature for 20 minutes

(15) Remove the aluminum foil and plate sealer. Add 100 µL of ELISA Stop Solution into each of the wells. Mix gently.

(16) Read the absorbance at 450 nm within 10 minutes in a microplate reader.

PROCEDURAL NOTES

1. It is recommended that all calibrators, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused antibody coated strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents should be mix gently and thoroughly prior use. Avoid foaming.

Quality Control

To assure the validity of the results each assay should include adequate controls with known anti-gliadin IgG levels. We recommend that all assays include the laboratory' s own controls in addition to those provided with this kit.

Calculation

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the calibrator 1 (0 U/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibrator curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

The gliadin IgG concentrations for the controls and samples are read directly from the calibrator curve using their respective corrected absorbance. If log-log graph paper or computer assisted data reduction program utilizing logarithmic transformation are used, sample having corrected absorbance between the 10 U/mL calibrator and the next highest calibrator should be calculated by the formula:

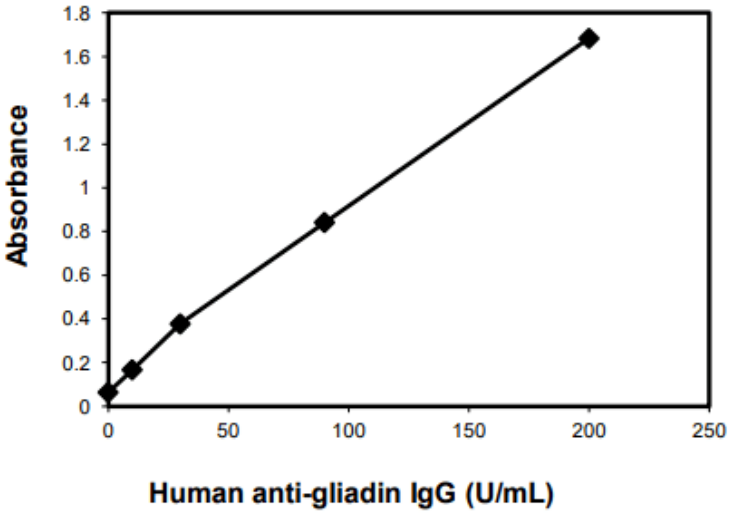
$$\text{Value of unknown} = \frac{\text{Corrected absorbance (unknown)}}{\text{Corrected Absorbance } (2^{nd}\text{ STD})} \times \text{Value of the } 2^{nd}\text{ STD}$$

Typical Standard Curve

A typical absorbance data and the resulting calibrator curve from human gliadin IgG ELISA are represented. This curve should not be used in lieu of calibrator curve run with each assay.

Well I.D.	OD 450 nm Absorbance			Results U/mL
	Readings	Average	Corrected	
0 U/mL	0.067 0.060	0.064	0.000	
10 U/mL	0.167 0.166	0.167	0.103	
30 U/mL	0.379 0.375	0.377	0.313	
90 U/mL	0.824 0.858	0.841	0.777	
200 U/mL	1.664 1.703	1.683	1.619	
Control 1	0.128 0.129	0.129	0.065	6.31 U/mL
Control 2	0.485 0.444	0.465	0.401	46.45 U/mL

Gliadin IgG EIA



Reference Values

Serum from 77 normal adults, 25 patients with confirmed celiac disease as well as 60 suspect patients were measured with this EIA. The following is a guide to interpretation of results. Because the prevalence of human anti-gliadin IgG antibodies may vary depending on a number of factors such as age, gender,

geographical location, race, type of test used and clinical history of individual patients, it is strongly recommended that each laboratory should establish its own "normal" range based on populations encountered.

< 30 U/mL: Negative

30 – 50 U/mL: Borderline

> 50 U/mL: Positive

Precision

Intra-assay precision:

Mean Gliadin IgG Value (U/mL)	CV (%)
40.8	3.4
174.1	4.8

Inter-assay precision:

Mean Gliadin IgG Value (U/mL)	CV (%)
41.2	6.8
172.6	5.3

Sensitivity

The sensitivity of this gliadin IgG EIA as determined by the 95% confidence limit on 20 duplicate determination of zero calibrator is about 1 U/mL.

Specificity

The microplates are coated with highly purified alpha gliadin. No cross-reactivity to other autoantibodies has been observed.

Linearity

Two human serum samples were diluted with assay buffer and assayed. The results in the value of U/mL are as follows:

#	DILUTION	OBSERVED VALUE	EXPECTED VALUE	RECOVERY %
1	1:100	180	-	-
	1:200	88.4	90.0	98
	1:400	45.7	45.0	102
	1:800	21.9	22.5	97
2	1:100	71.2	-	-
	1:200	35.1	35.6	99
	1:400	16.9	17.8	95
	1:800	8.1	8.9	91

Precautions

The reagents must be used in research laboratory and is for research use only. The source material for reagents containing bovine serum was derived in the contiguous 48 United States.

It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

Limitations

1. The results obtained with the anti-Gliadin IgG Test Kit should not be interpreted as diagnostic.
2. Gliadin IgA negative results in untreated patients does not rule out gluten-sensitive enteropathy when associated with high levels of gliadin IgG antibodies. The finding can often be explained by selective IgA deficiencies, a relative frequent finding in celiac disease.
3. Since there is no Gold Standard concentration available for gliadin IgG measurement, the values of assay calibrators were established and calibrated in arbitrary units (U/mL).
4. For unknown sample value read directly from the assay that is greater than 200 U/mL, it is recommended to measure a further diluted sample for more accurate measurement.
5. Bacterial or fungal contamination of serum specimens or reagents, or cross contamination between reagents may cause erroneous results.
6. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.
7. This instruction manual is for reference only. Please refer to the IFU upon arrival.