EPITOPE DIAGNOSTICS, INC.

EDI™ Human Anti-Gliadin IgA EIA Kit

Enzyme Immunoassay (EIA) for the measurement of human anti-gliadin immunoglobulin A (IgA) level in Serum

REF KT-830 CE IVD 96

INTENDED USE

This microplate based EIA (enzyme immunoassay) kit is intended for the quantitative determination of human anti-gliadin IgA level in serum. The assay is a useful tool in the aid of diagnosis of celiac disease. This kit is for in vitro diagnostic use only.

SUMMARY OF PHYSIOLOGY

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.

ASSAY PRINCIPLE

This EIA is designed, developed and produced for the quantitative measurement of human anti-gliadin IgA 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 IgA 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 IgA subclass specific antibody (tracer antibody) is added to each well. After an incubation period an immunocomplex of "gliadin – human anti-gliadin IgA – 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 IgA on the wall of the microtiter well is directly proportional to the amount of human anti-gliadin antibody level in the sample. A calibration 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 calibration curve.

REAGENTS: PREPARATION AND 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.

1. Gliadin Coated Microplate (30031)

Microplate coated with a highly purified Gliadin antigen.

Qty:	1 x 96 well microplate
Storage:	2 – 8°C
Preparation:	Ready to Use.

2.Gliadin hIgA Tracer Antibody (30050)

HRP-conjugated anti-human IgA tracer antibody in a stabilized protein matrix.

Qty:	1 x 0.6 mL
Storage:	2 – 8°C
Preparation:	21X Concentrate. This reagent must be diluted with Tracer Antibody Diluent (30052) before use.

3. Tracer Antibody Diluent (30052)

For antibody dilution.

Qty:	1 x 12 mL
Storage:	2 – 8°C
Preparation:	Ready to Use.

4. ELISA Wash Concentrate (10010)

Surfactant in a phosphate buffered saline with non-azide preservative.

Qty:	1 x 30 mL
Storage:	2 – 25°C
Preparation:	30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

5. ELISA HRP Substrate (10020)
Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

6. ELISA Stop Solution (10030)
0.5 M sulfuric acid
Qty: 1 x 12 mL
Storage: 2 –25°C
Preparation: Ready to Use.

7. Gliadin IgA Calibrators Levels 1 to 5 (30035 - 30039)
Human anti-Gliadin IgA in a liquid bovine serum albumin-based matrix with a non azide preservative. Refer to each vial for exact concentration.
Qty: 5 x Vials
Storage: <-20°C
Do not exceed 3 freeze-thaw cycles.
Preparation: Ready to Use

8. Gliadin IgA Controls (30040, 30041)
Human anti-Gliadin IgA in a liquid bovine serum albumin-based matrix with a non azide preservative. Refer to each vial for exact concentration.
Qty: 2 x Vials
Storage: <-20°C
Do not exceed 3 freeze-thaw cycles.
Preparation: Ready to Use

9. Patient Sample Diluent (30049)
Phosphate buffer with protein stabilizers and preservative.
Qty: 1 x 60 mL
Storage: 2 – 8°C
Preparation: Ready to Use

SAFETY PRECAUTIONS

The reagents are for in vitro diagnostic use only. Source material which contains reagents of bovine serum albumin 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 were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. 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.

MATERIALS REQUIRED BUT NOT PROVIDED

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 x 75 mm or 13 x 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.

SPECIMEN COLLECTION & STORAGE

Only 10 µL of human serum is required for gliadin IgA 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.

ASSAY PROCEDURE

1. Reagent Preparation

1. Prior to use allow all reagents to come to room temperature (20-25 ºC). Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate (10010) must be diluted to working solution prior use. Please see REAGENTS section for details.

2. Specimen Preparation

Patient sample needs to be diluted 1:101 with patient sample diluent (30049) before being measured

1. Label a test tube (12x75 mm).
2. Add 1 mL of patient sample diluentt (30049) to each tube. Pipette 10 µL of patient serum sample to the tube.

3. Assay Procedure

1. Place a sufficient number of microwell strips (30031) in a holder to run calibrators (30035 - 30039) and controls (30040, 30041), and samples in duplicate.
2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
B	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
C	Calibrator Level 2	Control 1	SAMPLE 3
D	Calibrator Level 2	Control 1	SAMPLE 3
E	Calibrator Level 3	Control 2	SAMPLE 4
F	Calibrator Level 3	Control 2	SAMPLE 4
G	Calibrator Level 4	SAMPLE 1	SAMPLE 5
H	Calibrator Level 4	SAMPLE 1	SAMPLE 5

3. Add **100 µL** of calibrators (30035 - 30039) and controls (30040, 30041), and samples into the designated microwells.
4. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **60 minutes.**
5. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
6. Prepare the antibody working solution by 1:21 fold dilution of the tracer antibody (30050) with the diluent (30052). For each strip, it is required to mix 1 mL of the tracer antibody diluent (30052) with 50 µL of the tracer antibody (30050) in a clean test tube.
   Note: This antibody working solution should be freshly prepared.

7. Add **100 µL** of the antibody working solution into each of the wells. Mix by gently tapping the plate.
8. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **30 minutes.**
9. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
10. Add **100 µL** of ELISA HRP Substrate (10020) into each of the wells. Mix by gently tapping the plate.
11. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
12. Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (10030) into each of the wells. Mix by gently tapping the plate.
13. Read the absorbance at **450 nm** within **10 minutes** with 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.

INTERPRETION OF RESULTS

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 calibration 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 IgA concentrations for the controls and samples are read directly from the calibration 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

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

LIMITATIONS OF THE PROCEDURE

1. The results obtained with the anti-Gliadin IgA Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
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 Calibrator concentration available for gliadin IgA 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.

QUALITY CONTROL

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

EXPECTED 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 IgA 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.

Unit Value	Interpretation
< 30 U/mL	Negative
30 – 50 U/mL	Borderline
> 50 U/mL	Positive

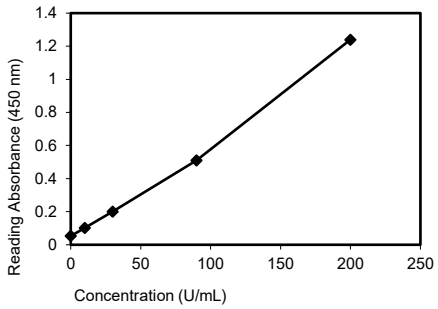
EXAMPLE DATA

A typical absorbance data and the resulting calibration curve from human Gliadin IgA ELISA are represented.

Note: This curve should not be used in lieu of calibration curve run with each assay.

Well ID	Reading Absorbance (450 nm)		Concentration (U/mL)
	Average	Corrected	
Calibrator Level 1: 0 U/mL	0.053	0.000	
Calibrator Level 2: 10 U/mL	0.101	0.048	
Calibrator Level 3: 30 U/mL	0.200	0.147	
Calibrator Level 4: 90 U/mL	0.520	0.467	
Calibrator Level 5: 200 U/mL	1.240	1.187	
Control 1	0.093	0.040	8.33
Control 2	0.293	0.240	46.25

Gliadin IgA EIA



PERFORMANCE CHARACTERISTICS

Sensitivity

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

Reproducibility and Precision

The intra-assay precision is validated by measuring two samples in a single assay with 20 replicate determinations. The inter-assay precision is validated by measuring two samples in duplicate in 12 individual assays. The results are as follows:

	Intra-Assay		Inter-Assay	
Sample	**1**	**2**	**1**	**2**
Mean (U/mL)	33.6	121.1	32.4	123.7
CV (%)	5.5	3.8	7.8	6.2

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 (U/mL)	Expected (U/mL)	Recovery (%)
Sample A	-	-	-
1:100	96.1	-	-
1:200	48.6	48.0	101
1:400	23.1	24.0	96
1:800	10.7	12.0	89
Sample B	-	-	-
1:100	68.4	-	-
1:200	33.6	34.2	97
1:400	16.2	17.1	95
1:800	9.1	8.6	106

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

This product is developed and manufactured by

Epitope Diagnostics, Inc.
7110 Carroll Road
San Diego, CA 92121, US

Please visit our website at www.epitopediagnostics.com to learn more about our products and services.

EC	REP

MDSS GmbH
Schiffgraben 41,
30175 Hannover, Germany

GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

IVD In Vitro Diagnostic Device	RUO For Research Use Only	LOT Lot Number
REF Catalog Number	Read instructions before use	Number of Tests
Store at	Use by	Keep away from heat and direct sun light
Manufacturer	EC REP Authorized Representative in Europe	CE European Conformity

SHORT ASSAY PROCEDURE

1. Add **100 µL** of the calibrators, controls, and diluted samples into the designated microwells.
2. Mix, cover, and incubate at **room temperature (20-25 ºC)** for **60 minutes**.
3. Wash each well five times.
4. Add **100 µL** of the antibody working solution into the designated microwells.
5. Mix, cover, and incubate at **room temperature (20-25 ºC)** for **30 minutes**.
6. Add **100 µL** of substrate to each well.
7. Cover and incubate at **room temperature (20-25 ºC)** for **20 minutes**.
8. Add **100 µL** of the stop solution to each well.
9. Read the absorbance at **450 nm.**