GliaDea IgA ELISA

Catalog Number:
GDA31-K01

Enzyme immunoassay for the determination of IgA antibodies against deamidated gliadin in human serum or plasma

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v. 1.0

INTENDED USE

The Eagle Biosciences GliaDea IgA ELISA Assay Kit is used for the quantitative determination of IgA antibodies against deamidated gliadin in human serum or plasma for the diagnosis of celiac disease.

Celiac disease (CD, sprue, gluten enteropathy) is an intolerance to gluten, a cereal protein present primarily in wheat. This gluten-induced enteropathy leads to a severe damage of the small intestine and a so-called "flat" mucosa. Due to this extensive lesions mal-absorption occurs accompanied with a depletion of key nutrients.

Gliadin the alcohol soluble fraction of gluten represents the causative agent of celiac disease. During resorption gliadin is deamidated by tissue transglutaminas (tTG). Linked to specific genetic predisposition parts of this deamidated gliadin activate HLA-DQ2 and -DQ8 antigen presenting cells. These provoke an inflammatory process in the small intestine triggering both cellular and humoral immune responses. Hence pathogenic Tissue damage as well as production of antibodies against deamidated gliadin and tTG occurs.

Celiac disease is one of the most common enteropathies found in infants, showing first symptoms already at the age between sixth and eighteen months. Incidence rates for celiac disease range from 1 in 300 (Western Ireland) to 1 in 4700 in European countries. However, a high number of subclinical cases of celiac disease have been detected by in-vitro tests revealing a prevalence of 4 in 1000. Individuals suffering from prolonged celiac disease additionally face an elevated risk of developing T cell lymphoma.

LITERATURE

Lerner A, Kumar V, Iancu TC: Immunological diagnosis of childhood coeliac disease: comparison between antigliadin, antireticulin and antiendomysial antibodies. Clin Exp Immunol 1994; 95:78-82

Schuppan D: Current concepts of celiac disease pathogenesis. Gastroenterology. 2000;119:234-42.

Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schup¬pan D: Identification of tissue transglutaminase as the autoantigen of coe¬liac disease. Nature Med 1977, 3:797-801

PRINCIPLE OF THE TEST

The Eagle Biosciences GliaDea IgA is an enzyme immunoassay for the quantitative determination of IgA antibodies to deamidated gliadin in human serum or plasma.

The antibodies of the calibrators, positive control, and diluted patient samples react with fragments of deamidated gliadin immobilized on the solid phase of microtiter plates. After an incubation period of 60 min at room temperature (18…25°C), unbound serum components are removed by a wash step.

The bound autoantibodies react specifically with anti-human-IgA-antibodies conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at room temperature. Excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. This enzyme reaction is stopped by dispensing an acidic solution (H_2SO_4) into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the concentrations of the antibodies of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve. Evaluating the test by a semi-quantitative method is also possible.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic and contaminated samples should not be used.

Samples are stable up to 3 days at 2-8°C, for extended storage freeze at -20 °C. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: ***Patient samples have to be diluted 1 + 100 (v/v),***
e.g. 10 µl sample + 1.0 ml sample diluent (C), prior to assay.

TEST COMPONENTS FOR 96 DETERMINATIONS

A Ag 96	**Microtiter plate**, 12 breakable strips per 8 wells (total 96 individual wells) coated with deamidated gliadin	1 vacuum sealed with desiccant
B BUF WASH 10x	**Concentrated wash buffer** sufficient for 1000 ml solution	100 ml concentrate capped white
C DIL	**Sample diluent**	100 ml ready for use capped black
D CONJ	**Conjugate** containing anti-human-IgA- (sheep) coupled with HRP	15 ml ready for use capped purple
E SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
F H2SO4	**Stop solution** 0.25 M sulfuric acid 0.25M	15 ml ready for use capped yellow
0 - 4 CAL	**Calibrators** (diluted serum) conc.: 1, 10, 30, 100, 300 U/ml	1 ml each ready for use capped white
P CONTROL	**Positive control** (diluted serum) conc.: see leaflet enclosed +	1 ml ready for use capped red
N CONTROL	**Negative control** (diluted serum) conc.: see leaflet enclosed -	1 ml ready for use capped green

Materials required in addition

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- distilled or de-ionized water

Size and storage

The Eagle Biosciences GliaDea IgA ELISA Assay Kit has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the GliaDea IgA have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water.
For example, dilute 8 ml of the concentrate with 72 ml of distilled water. The wash solution prepared is stable up to 30 days at 2 - 8 °C.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- **Dilute patient sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl serum + 1.0 ml of sample diluent (C)**.
- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature (18…25°C) before use. Mix gently, avoid foam.
2. Dispense **100 µl** calibrators (0 optional) 1 - 4 (quantitative) or **100 µl** calibrator 1 (semi-quantitative)**100 µl** control P (N optional) **100 µl** diluted patient samplesinto the respective wells.
3. Seal plate, incubate **60 min** at room temperature.
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Seal plate, incubate **30 min** at room temperature.
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **15 min** protected from light at room temperature.
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

DATA PROCESSING

GliaDea IgA screen allows both the quantitative (4 + 1 calibrators) and semi-quantitative (calibrator 1 for cut-off determination) evaluation of the results.

Quantitative evaluation

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 4 (CAL 0 optionally) on the ordinate, y-axis, (lin. scale) versus their respective antibody concentrations on the abscissa, x-axis, (log. scale). GliaDea IgA concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

Semi-quantitative evaluation

Results can be calculated semi-quantitatively using the binding index BI (ratio) between the optical density of an unknown sample and the optical density of calibrator 1 (10 U/ml):

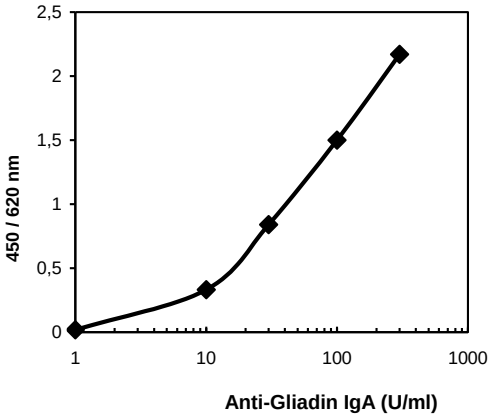
$$\mathbf{BI = OD_{Sample} / OD_{Calibrator\ 1}}$$

Both evaluation variants of GliaDea IgA may be achieved also with computer assisted analysis software intergrated in the photometers.

Example of typical assay results (quantitative)

well	OD (a)	OD (b)	OD(mean)	U/ml
Calibrator 0	0.020	0.017	0.019	**1**
Calibrator 1	0.326	0.338	0.332	**10**
Calibrator 2	0.829	0.853	0.841	**30**
Calibrator 3	1.478	1.520	1.499	**100**
Calibrator 4	2.184	2.158	2.171	**300**
Patient 1	1.192	1.204	1.198	**60**

TYPICAL STANDARD CURVE



Test validity

The test run is valid if:

- the mean OD of the calibrator 4 is ≥ 1.2
- Concentration of Control P see leaflet enclosed
- Control N is negative

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

REFERENCE VALUES

GliaDea IgA	quantitative (U/ml)	semi-quant. BI
negative	**< 10**	**< 1.0**
positive	**> 15**	**> 1.5**
grey zone	**10 – 15**	**1.0 – 1.5**

Specimens with concentrations detected in the grey zone should be tested again.

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-deamidated gliadin IgA levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values only provide a guide to values which might be expected.

Limitations of Method

Healthy individuals should be tested negative by the GliaDea IgA. However, asymptomatic patients might attain positive results.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

CHARACTERISTIC ASSAY DATA

Calibration

Due to the lack of an international reference material the GliaDea IgA is calibrated in arbitrary units (U/ml).

Diagnostic specificity and sensitivity

Assay data for GliaDea IgA were determined by testing of sera from 45 patients suffering from celiac disease and 64 blood donors. A test **sensitivity of 75.6 %** and a test **specificity of 100 %** were determined. In comparison the IgA ELISA using whole gliadin showed a sensitivity of 66.7 % and a specificity of 100 %.

Comparison of Competitor's assay

The serum panel listed above was also tested in comparison to a competitor's assay (IgA against deamidated gliadin), showing a sensitivity of 77.8 % and a specificity of 93.8 % for this assay.

Examinating the 45 sera of patients suffering from celiac disease a consensus of 84.4% could be found between both tests.

		Competitor's assay positive	Competitor's assay negative
GliaDea	**IgA**	31	3
GliaDea	**IgA**	4	7

The analysis of the 64 blood donors showed a total agreement of 93.8% between both kits.

		Competitor's assay positive	Competitor's assay negative
GliaDea	**IgA**	0	0
GliaDea	**IgA**	4	60

Precision

Intraassay (n = 20)		Interassay (n = 5 x 10)	
mean (U/ml)	**CV %**	**mean (U/ml)**	**CV %**
198.3	4.5	197.9	1.7
65.2	2.4	64.6	0.5
19.9	3.2	20.8	4.9

INCUBATION SCHEME

Dilute patients sample★ 10 µl sample + 1.0 ml sample diluent (C)

1	Bring all ready for use reagents to room temperature (18...25°C) before use.			
		calibrators	control	sera
2	Pipette Calibrators (0 - 4) or Calibrator 1 Controls (P, N) prediluted 1 + 100 patient sera	100 µl	100 µl	100 µl
3	Incubate	60 minutes at room temperature		
4	Wash	Decant, dispense 3 x 300 µl (made of B)		
5	Pipette conjugate (D)	100 µl	100 µl	100 µl
6	Incubate	30 minutes at room temperature		
7	Wash	Decant, dispense 3 x 300 µl (made of B)		
8	Pipette substrate (E)	100 µl	100 µl	100 µl
9	Incubate protected from light	15 minutes at room temperature		
10	Pipette stop solution (F)	100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm			

SAFETY PRECAUTIONS

- **This Gliadin IgA ELISA Assay Kit is for research use only**. Follow the working instructions carefully. This instruction manual is valid only for the present kit with the given composition.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Neolone M10 (< 0.1 % w/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.