



INTENDED USE

Anti-Gliadin IgA is used for the quantitative determination of IgA antibodies to gliadin in human serum for the diagnosis of celiac disease.

Celiac disease, or gluten-sensitivity, is found already in neonates and is characterized by small intestinal damages leading to a so-called "flat" mucosa. Due to this extensive lesions mal-absorption occurs frequently accompanied with a depletion of key nutrients.

Gliadin the alcohol soluble fraction of gluten represents the causative agent of celiac disease that provokes an inflammatory process in the small intestine. Gliadin is a substrate of tissue transglutaminase and cross-linked into high molecular complexes triggering probably both cellular and humoral immune responses.

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.

Today diagnosis of celiac disease comprises small intestine biopsy demonstrating a "flat" mucosa prior to a gluten-free diet and the following reconstitution of the mucosa after onset of the diet. Determination of anti-gliadin IgG and IgA by ELISA as well as the detection of anti-endomysium IgA by immunofluorescence has been considered as the main serological parameters for celiac disease so far.

Generic Assays offers a complete range of serological markers for celiac disease including Anti-Gliadin IgG, Anti-Gliadin IgA and as well Anti-huTransG and Anti- hu tTG IgG, ELISAs for the determination of IgA and IgG autoantibodies to tissue transglutaminase. All assays employ the same assay scheme and predilution maximizing laboratory efficiency.

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

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

# Anti-Gliadin IgA

- 96 determinations -



IVD In vitro diagnostic device

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

<b>REF</b>	Catalogue number	<b>LOT</b>	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk

PRINCIPLE OF THE TEST


Anti-Gliadin IgA is an enzyme immunoassay for the quantitative determination of IgA antibodies to gliadin.

The antibodies of the calibrators, positive control, and diluted patient samples react with gliadin immobilized on the solid phase of microtiter plates. Highly purified gliadin coated on the microtiter plate guarantees the specific binding of gliadin IgA antibodies of the specimen under investigation. Following 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<sub>2</sub>SO<sub>4</sub>) 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.



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## 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.

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.*

The samples may be kept at 2 - 8 °C for up to two days. Long-term storage requires -20 °C.

## TEST COMPONENTS FOR 96 DETERMINATIONS

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

### Materials required in addition

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
- pipette tips
- trough for multi-channel pipette
- 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
- graduated cylinders
- tubes (2 ml) for sample preparation
- distilled or de-ionized water

### Size and storage

Anti-Gliadin IgA 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 Anti-Gliadin 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 - 4 (quantitative) or **100 µl** of calibrator 1 (semi-quantitative) **100 µl** positive control **100 µl** diluted patient samples into 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

Anti-Gliadin IgA allows both the quantitative (4 calibrators) and semi-quantitative (calibrator 1) 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 anti-gliadin concentrations on the abscissa, x-axis, (log. scale). Anti-gliadin 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):

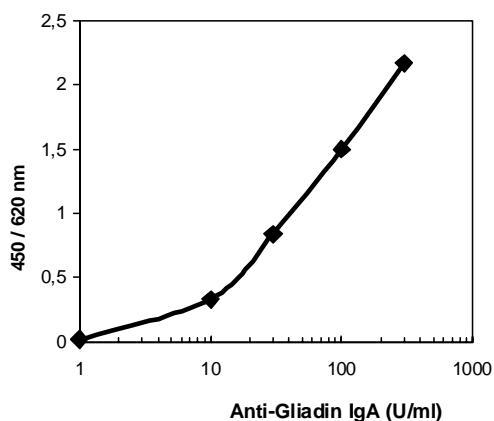
$$BI = OD_{\text{Sample}} / OD_{\text{Calibrator 1}}$$

Both evaluation variants of Anti-Gliadin 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 calibrators 1 is  $\leq 0.5$
- the mean OD of the calibrators 4 is  $\geq 1.2$

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

Anti-Gliadin 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-Gliadin IgA levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

### Limitations of Method

Healthy individuals should be tested negative by the Anti-Gliadin IgA. However, anti-Gliadin IgA antibody positive apparently healthy persons do occur.

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 Anti-Gliadin IgA is calibrated in arbitrary units (U/ml).

### Linearity

Dilutions of selected positive specimens in anti-Gliadin IgA free human serum are determined according to their expected theoretical values with Anti-Gliadin IgA.

### Analytical Sensitivity

The analytical sensitivity of the Anti-Gliadin IgA is 5 U/ml.

### Diagnostic specificity and sensitivity

ROC analysis has been performed for Anti-Gliadin IgA measuring sera from 73 patients suffering from celiac disease, 28 patients with other diseases and 317 healthy blood donors.

Using a cut-off value of 15 U/ml the specificity was determined 96.5 % and the sensitivity 81.7 %.

### Precision

Intraassay ( n = 8 )		Interassay ( n = 4 x 8 )	
mean (U/ml)	CV %	mean (U/ml)	CV %
128	2,43	120	3,91
86	3,72	76	6,15
25	2,80	23	4,32
18	1,10	18	4,83

### Clinical Evaluation

In a clinical study 171 sera were compared in Anti-Gliadin IgA and in a competitor's assay.

	Competitor's assay positive	Competitor's assay negative
Anti-Gliadin IgA positive	79	1
Anti-Gliadin IgA negative	1	90

