

**Revised 29 July 2010 rm (Vers. 2.1)**

*Please use only the valid version of the package insert provided with the kit.*

## **1 INTENDED USE**

The Anti-Gliadin IgG kit is an indirect solid phase enzyme immunometric assay (ELISA) designed for the measurement of IgG class antibodies directed against deamidated Gliadin peptides (DGP) in human serum.

### **1.1 CLINICAL SIGNIFICANCE**

Celiac disease, also known as gluten sensitive enteropathy is primarily a disease of the infant organism. It is caused by a hypersensitivity reaction in response to gliadin, a protein being present in many cereals. This, non IgE mediated food allergy leads to massive malabsorption disturbances and is characterized by a complete atrophy of the villi and a hyperplasia of the crypts of the upper intestine.

Gliadins are proteins containing high amounts of the amino acids prolin and glutamine. This protein belongs to the nutritive tissue of the grain seeds of wheat, oat, barley and rye and is responsible for the baking properties of the flour.

Due to the possibilities of the highly specific and sensitive serological determination of IgA and IgG antibodies against gliadin the invasive procedures of biopsies can be given up. In the past several biopsies have been done with patients when celiac disease was suspected, after a period of a gluten-free diet and also after a specific gluten challenge Gliadin antibodies titer correlate very well with the morphological appearance of the mucosa of the upper intestine. It has been well documented that gliadin antibodies level fall very quickly after a gluten free diet has begun and rise immediately after restoring gluten to the diet. Thus the serological test represents a reliable method to monitor patients, and in particular children and teenagers, for their adherence to the gluten-free diet.

## **2 PRINCIPLE**

The anti-gliadin IgG test is based on the binding of present antibodies in calibrators, controls or prediluted patient samples on the synthetic deamidated gliadin peptides (DPG) coated on the inner surface of the wells. After a 30 minutes incubation the microplate is washed with wash buffer for removing non-reactive serum components.

An anti-human-IgG horseradish peroxidase conjugate solution recognize IgG class antibodies bound to the immobilized antigens. After a 30 minutes incubation any excess enzyme conjugate, which is not specifically bound is washed away with wash buffer.

A chromogenic substrate solution containing TMB is dispensed into the wells. After 15 minutes of incubation the color development is stopped by adding the stop solution. The solutions color change into yellow. The amount of color is directly proportional to the concentration of IgG antibodies present in the original sample.

## **3 REAGENT, MATERIAL AND INSTRUMENTATION**

### **3.1 Reagents and material supplied in the kit**

1. **Anti-Gliadin Standards S0 – S4;** 5 x (1 vial = 1.2 mL)  
Phosphate buffer 0.1 M, NaN<sub>3</sub> < 0.1%
2. **Control** 2 x , negative / positive (1 vial = 1.2 mL)  
Phosphate buffer 0.1 M, NaN<sub>3</sub> < 0.1%
3. **Sample Diluent** (1 bottle = 100 mL)  
Phosphate buffer 0.1 M, NaN<sub>3</sub> < 0.1%

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4. **Enzyme Conjugate** (1 bottle = 15 mL)  
Anti h-IgG conjugated with peroxidase, BSA 0.1%, Proclin < 0.0015%
5. **Coated Microplate** (1 microplate breakable)
6. **TMB-Substrate Solution** (1 bottle = 15 mL)  
3,3',5,5'-tetramethylbenzidine 0.26 g/L, hydrogen peroxide 0.05%, Proclin < 0.0015%
7. **Wash Solution Concentrate** (1 bottle = 50 mL)  
Phosphate buffer 0.2M, Proclin < 0.0015%
8. **Stop Solution** (1 bottle = 15 mL)  
Sulfuric acid 0.15 M

**3.2 Reagents necessary not supplied**

Distilled water.

**3.3 Auxiliary materials and instrumentation**

Automatic dispenser.

Microplates reader

**4 PRECAUTION**

- This kit is intended for in vitro use by professional persons only.
- Please adhere strictly to the sequence of pipetting steps provided in this protocol.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- All reagents should be stored refrigerated at 2 - 8 °C in their original container.
- Do not interchange kit components from different lots. The expiration dates stated on the labels of the shipping container and all vials have to be observed. Do not use kit components beyond their expiration dates.
- Allow all kit components and specimen to reach room temperature prior to use and mix well.
- All human source material used in the preparation of standards and controls for this product has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV by FDA cleared methods. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Positive and Negative Control should be handled in the same manner as potentially infectious material.
- Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperatures may initiate spontaneous combustion.

**5 STORAGE CONDITION**

- Store all the kit reagents at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.

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## **6 PROCEDURE**

### **6.1 Preparation of the Standard (S0 – S4)**

Since no international reference preparation for Anti-Gliadin antibodies is available, the assay system is calibrated in relative arbitrary units (AU). The standards have approximately the following concentration:

	S0	S1	S2	S3	S4
AU/mL	0	15	30	60	240

### **6.2 Preparation of the Sample**

For determination of Anti-Gliadin serum or plasma are the preferred sample matrixes.

All serum and plasma samples have to be prediluted with sample diluent 1 : 100.

Therefore 10 µL of sample may be diluted with 1,000 µL of sample diluent.

No special preparations are necessary. Collect blood by venipuncture into vacutainers and separate serum or plasma from the cells by centrifugation after clot formation.

Samples may be stored refrigerated at 2 - 8 °C for at least 5 days. For longer storage of up to six months samples should be stored frozen at -20 °C. To avoid repeated thawing and freezing the samples should be aliquoted.

Neither Bilirubin nor Hemolysis have significant effect on the procedure.

### **6.3 Preparation of the Wash Solution**

Dilute the contents of each vial of the buffered wash solution concentrate (10x) with distilled water to a final volume of 500 ml prior to use.

For smaller volumes respect the 1:10 dilution ratio.

The diluted wash solution is stable for 30 days at 2-8°C.

### **6.4 Procedure**

Allow all reagents to stand at room temperature (22-28°C).

As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S0-S4), two for each control, two for each sample and one for Blank.

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Reagent	Standard	Sample	Blank
Standard S0-S4	100 µL		
Controls	100 µL		
Sample		100 µL	
Incubate 30 minutes at room temperature (22-28°C). Remove the contents from each well, wash the wells three times with 300 µL diluted wash solution			
Conjugate	100 µL	100 µL	
Incubate 30 minutes at room temperature (22-28°C). Remove the contents from each well, wash the wells three times with 300 µL diluted wash solution.			
TMB substrate	100 µL	100 µL	100 µL
Incubate 15 minutes in the dark at room temperature (22-28°C).			
Stop solution	100 µL	100 µL	100 µL
Read the absorbance (E) at 450 nm against Blank.			

## 7 RESULTS

### 7.1 Standard curve

For Anti-Gliadin IgG a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed-Spline Approximation and log-log coordinates are also suitable.

#### Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

#### TYPICAL RESULTS (example only)

The figures below show typical results for Anti- Gliadin IgG. These data are intended for illustration only and should not be used to calculate results from another run.

N	OD1	OD2	Mean OD	Conc. 1	Conc. 2	Mean Conc.	CV %
<b>STD0</b>	0.011	0.011	0.011	0.09	0.09	0.09	6E-7
<b>STD1</b>	0.165	0.161	0.163	14.74	14.36	14.55	1.89
<b>STD2</b>	0.322	0.324	0.323	30.46	30.67	30.57	0.48
<b>STD3</b>	0.590	0.590	0.590	59.78	59.78	59.78	4E-7
<b>STD4</b>	1.694	1.768	1.731	232.1	248.1	240.1	4.71

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## **8 WASTE MANAGEMENT**

Reagents must be disposed off in accordance with local regulations.

## **9 BIBLIOGRAPHY**

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