DRG® Gliadin IgA (Anti) (EIA-5081)

USA: RUO

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 IgA kit is an indirect solid phase enzyme immunometric assay (ELISA) designed for the measurement of IgA 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.

2 PRINCIPLE

The anti-gliadin IgA 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-IgA horseradish peroxidase conjugate solution recognize IgA 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 IgA 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_3 < 0.1%
2. **Control** 2 x , negative / positive (1 vial = 1.2 mL)
   Phosphate buffer 0.1 M, NaN_3 < 0.1%
3. **Sample Diluent** (1 bottle = 100 mL)
   Phosphate buffer 0.1 M, NaN_3 < 0.1%
4. **Enzyme Conjugate** (1 bottle = 15 mL)
   Anti h-IgA 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 research 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.

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.

The patients need not to be fasting, and 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.

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 IgA 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 IgA. 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 %
STD0	0,013	0,009	0,011	0,18	0,00	0,09	141,42
STD1	0,205	0,206	0,206	14,93	15,00	14,96	0,36
STD2	0,401	0,405	0,403	29,89	30,20	30,04	0,73
STD3	0,794	0,770	0,782	60,96	59,01	59,99	2,30
STD4	2,558	2,710	2,634	231,2	249,0	240,1	5,26

8 WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

9 BIBLIOGRAPHY

1. Chartrand LJ, Seidman EG. Celiac disease is a lifelong disorder. Clin.Invest.Med., Vol. 19, 357-361, 1996
2. Cornell HJ. Coeliac disease: A review of the causative agents and their possible mechanisms of action. Amino Acids, Vol. 10, 1-19, 1996
3. Cronin CC, Feighery A, Ferriss JB, Liddy C, Shanahan F, Feighery C. High prevalence of celiac disease among patients with insulin-dependent (type I) diabetes mellitus. Am.J Gastroenterol., Vol. 92, 2210-2212, 1997
4. Jokinen J, Peters U, Maki M, Miettinen A, Collin P. Celiac sprue in patients with chronic oral mucosal symptoms. J Clin.Gastroenterol., Vol. 26, 23-26, 1998
5. Taminiau JA. Celiac disease. Curr.Opin.Pediatr., Vol. 8, 483-486, 1996
6. Williams CN. Celiac disease: past, present and future. Can.J Gastroenterol., Vol. 11, 647-649, 1997