Anti Deamidated Gliadin Peptide(DGP) IgA

GWB-521208

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FOR RESEARCH USE ONLY

Enzyme immunoassay for the quantitative determination of auto-antibodies against deamidated Gliadin peptides (DGP) in human serum or plasma

1. INTENDED USE

Anti-Deamidated Gliadin Peptide (DPG) IgA kit is a solid phase enzyme immunometric assay (ELISA) designed for the quantitative measurement of IgA class antibodies directed against deamidated Gliadin peptides (DGP) in human serum or plasma. Anti-Deamidated Gliadin Peptide (DPG) IgA is intended for research use only.

2. PRINCIPLE OF THE ASSAY

Anti-Deamidated Gliadin Peptide (DPG) IgA test is based on the binding of present antibodies in calibrators, controls or prediluted samples on the syntetic deamidated Gliadin peptides (DGP) coated on the inner surface of the wells. After a 30 minutesincubation the microplate is washed with wash buffer for removing non-reactive serum components.An anti-human-IgA horseradish peroxidase conjugate solution recognizes 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 developmentis stopped by adding the stop solution. The solutions color changes into yellow. The amount of color is directly proportional to theconcentration of IgA antibodies present in the original sample.

3. MATERIALS

3.1. Reagents supplied

Anti-DGP Coated Wells: 12 breakapart 8-well snap-off strips coated with DGP; in resealable aluminium foil.
Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.15 mol/l (avoid any skin contact), ready to use
Conjugate: 1 bottle containing 15ml Anti h-IgA conjugate with horseradish peroxidise (HRP), BSA 0,1%, Proclin < 0,0015%
TMB Substrate Solution: 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine 0,26 g/L, hydrogen peroxide 0,05%
Wash solution: 1 bottle containing 50 ml (10x conc.) Phosphate buffer 0,2M, proclin < 0,0015%
Sample Diluent : 1 bottle containing 100 ml Phosphate buffer 0,1M, NaN3 < 0,1%
Anti-DGP Standards: 5 bottles, 1,2 ml each, ready to use
Standard 0: 0 AU/ml
Standard 1: 15 AU /ml
Standard 2: 30 AU /ml
Standard 3: 60 AU /ml
Standard 4: 240 AU /ml
Positive Control: 1 bottle containing 1,2 ml,Phosphate buffer 0,1M,NaN_3 < 0,1%, human serum,ready to use
Negative Control: 1 bottle containing 1,2 ml,Phosphate buffer 0,1M,NaN_3 < 0,1%, human serum,ready to use

3.2. Materials supplied

1 Strip holder
1 Cover foils
1 Test protocol
1 Distribution and identification plan

3.3. Materials and Equipment needed

ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
Manual or automatic equipment for rinsing wells
Pipettes to deliver volumes between 10 and 1000 µl
Vortex tube mixer
Distilled water
Disposable tubes
Timer

4. STABILITY AND STORAGE

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 antigen coated microwell strips should be resealed securely in the foil pouch containing desiccants and stored at 2-8°C.

5. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards to room temperature (22…28°C) before starting the test run!

5.1. Coated snap-off Strips

The ready to use break apart snap-off strips are coated with DGP. Store at 2…8 °C. Open the bag only when it is at room temperature.
Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2…8 °C; stability until expiry date. Do not remove the adhesive sheets on the unused strips.

5.2. Anti-DGP Standards / control

Since no international reference preparation for Anti-DGP antibodies is available, the assay system is calibrated in relative arbitrary units. The standards have approximatively the following concentration:

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

5.3. TMB Substrate Solution

The bottle contains 15 ml of 3,3',5,5'-tetramethylbenzidine 0,26 g/L, hydrogen peroxide 0,05%. The reagent is ready to use and has to be stored at 2...8°C in the dark. *The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away*.

5.4. Stop Solution

The bottle contains 15 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.

5.5. Wash Solution

Dilute the content 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.

5.6. Sample Diluent

The bottle contains 100 ml Phosphate buffer 0,1M, NaN3 < 0,1%

5.7. Conjugate IgA

The bottle containing 15ml Anti h-IgA conjugate with horseradish peroxidise (HRP), BSA 0,1%, Proclin < 0,0015%

6. SPECIMEN COLLECTION AND PREPARATION

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

All serum and plasma samples have to be prediluted with sample diluent 1 : 100. Therefore 10 L of sample may be diluted with 990L of sample diluent. No special preparations are necessary. Collect blood by venipuncture into vacutainers and
separate serum (after clot formation) or plasma from the cells by centrifugation.

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 storedfrozen at -20°C. To avoid repeated thawing and freezing the samples should be aliquoted.Neither Bilirubin nor Hemolysis have significant effect on the procedure.The Controls are ready to use.

7. ASSAY PROCEDURE

7.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Please allocate at least:

1 well (e.g. A1) for the substrate blank 2 wells (e.g. B1+C1) for

standard 0 2 wells (e.g. D1+E1) for standard 1 2 wells (e.g. F1+G1) for standard 2 2 wells (e.g. H1+A2) for standard 3 2 wells (e.g. B2+C2) for standard 4 2 wells (e.g. D2+E2) for positive control 2 wells (e.g. F2+G2) for negative control

It is *recommended to determine standards and samples in duplicate.*Perform all assay steps in the order given and without any appreciable delays between the steps.A clean, disposable tip should be used for dispensing each standard and each sample.

7.2. Test Procedure

Reagent	Standard	Sample or Controls	Blank
Standard S0-S4	100 µL		
Controls		100 µL	
Diluted 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 of 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 of 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
Shake the microplate gently Read the absorbance (E) at 450 nm against Blank.			

8. RESULTS

For Anti-DGP IgA a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method ofchoice. Smoothed-Spline Approximation and log-log coordinates are also suitable. However, we recommend using a Lin-Log curve.First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density ofeach calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibratorpoints may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibrationcurve by interpolation.

TYPICAL RESULTS (example only)The figures below show typical results for Anti-DGP IgA. These data are intended for illustration only and should not be used tocalculate results from another run.

N	OD1	OD2	mean	C1	C2	mean	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

9. SPECIFIC PERFORMANCE CHARACTERISTICS

9.1. Precision

Intra-Assay Within run variation was determined by replicate 16 times two different sera with values in the range of standard curve. The within assay variability is ≤5.8%

Inter-Assay Between run variation was determined by replicate the measurements of two different control sera with different lots of kits and/or different mix of lots of reagents. The between assay variability is ≤10.2%.

9.2. Sensitivity

Comparison test against a commercial reference kit, performed on 69 sera (29 of them positive sera and 40 negative sera) shows a 100% sensitivity.

9.3. Specificity

Comparison test against a commercial reference kit, performed on 69 sera (29 of them positive sera and 40 negative sera) shows a 97.6% specificity

9.4. Detection limit

The lowest concentration of anti-DGP that can be distinguished from zero standard is 0.12 AU/mL with a confidence limit of 95%. The lowest concentration of anti-DGP IgA that can be distinguished from zero standard is less than 0.74 AU/mL with a confidence limit of 95%.

10. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

11. WARNINGS AND PRECAUTIONS

This kit is intended for research use only by professional persons. Use appropriate personal protective equipment while working with the reagents provided.All human source material used in the preparation of standards and controls for this product has been tested and found negative forantibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or otherinfectious agents are absent. Therefore, the Standard and the Controls should be handled in the same manner as potentially infectiousmaterial.Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine proteinhas been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.Some reagents contain small amounts of Sodium Azide (NaN3) or Proclin 300R as preservatives. Avoid the contact with skin ormucosa.Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing toform potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water toprevent azide build-up.The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury,avoid inhalation, ingestion or contact with skin and eyes.The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested.To prevent chemical burns, avoid contact with skin and eyes.Avoid the exposure of reagent TMB/H2O2 to directed sunlight, metals or oxidants.PRECAUTIONSPlease adhere strictly to the sequence of pipetting steps provided in this protocol.All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated.Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.Do not interchange kit components from different lots. The expiry dates printed on the labels of the box and of the vials must beobserved. Do not use any kit component beyond their expiry date.WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if notused properly; therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exactamount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, for doses dispensed withthe aid of automatic and semi-automatic devices, before using the conjugate, it is advisable to clean the fluid handling system,ensuring that the procedures of washing, deproteinization and decontamination are effective in avoiding contamination of theconjugate; this procedure is highly recommended when the kit is processed using analyzers which are not equipped with disposabletips.If you use automated equipment is your responsibility to make sure that the kit has been appropriately tested.The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extendbeyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than oneplate is used, it is recommended to repeat the dose response curve in each plateAddition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution.Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during thereaction.Observe the guidelines for performing quality control in laboratories by assaying controls and/or pooled sera.Maximum precision is required for reconstitution and dispensation of the reagents.Samples microbiologically contaminated should not be used in the assay. Highly lipemeic or haemolysed specimens should similarlynot be usedPlate readers measure vertically. Do not touch the bottom of the wells.

12. BIBLIOGRAPHY

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SCHEME OF THE ASSAY

Anti-DGP

Test Preparation

Prepare reagents and samples as described.Establish the distribution and identification plan for all specimens and controls on the resultsheet supplied in the kit.

Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

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