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Anti Deamidated Gliadin Peptide (DGP) IgA

for routine analysis

Quantitative determination of IgA class antibodies against deamidated Gliadin peptides (DGP) in human serum or plasma

RUO | LOT | See external label | 2°C 8°C | Σ = 96 tests | REF DKO107

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.

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.
Accordingly patients suffering from celiac disease must maintain a gluten free diet for the rest of their life.
Gliadins are proteins containing high amounts of the amino acids prolin and glutamine. These proteins belong to the nutritive tissue of the grain seeds of wheat, oat, barley and rye and are 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 DGP 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. DGP antibodies titer has been proved to correlate very well with the morphological appearance of the mucosa of the upper intestine. It has been well documented that DGP 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

Anti Deamidated Gliadin Peptide (DPG) IgA test is based on the binding of present antibodies in calibrators, controls or prediluted patient samples on the syntetic deamidated Gliadin peptides (DGP) 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 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 development is stopped by adding the stop solution. The solutions color changes into yellow. The amount of color is directly proportional to the concentration of IgA antibodies present in the original sample.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. Calibrators (5 vials, 1.2 mL each)
Phosphate buffer 0.1 M, NaN_3 < 0.1%, human serum

CAL0	REF DCE002/10706-0
CAL1	REF DCE002/10707-0
CAL2	REF DCE002/10708-0
CAL3	REF DCE002/10709-0
CAL4	REF DCE002/10710-0

2. Controls (2 vials, 1.2 mL each, ready to use)
Phosphate buffer 0.1 M, NaN_3 < 0.1%, human serum

Negative Control	REF DCE045/10701-0
Positive Control	REF DCE045/10702-0

3. Sample Diluent (1 vial, 100 mL)
Phosphate buffer 0.1 M, NaN_3 < 0.1%
REF DCE053-0

4. Conjugate (1 vial, 15 mL)
Anti h-IgA conjugated with horseradish peroxidise (HRP), BSA 0.1%, Proclin < 0.0015% REF DCE002/10702-0

5. Coated Microplate (1 breakable microplate)
DGP coated on the microplate REF DCE002/10703-0

6. TMB Substrate (1 vial, 15 mL)
H_2O_2 -TMB (0,26 g/L) *(avoid any skin contact)*
REF DCE004-0

7. Stop Solution (1 vial, 15 mL)
Sulfuric acid 0.15M *(avoid any skin contact)*
REF DCE005-0

8. 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0.2M, pH 7.4 **REF DCE054-0**

3.2. Reagents necessary not supplied
Distilled water.

3.3. Auxiliary materials and instrumentation
Automatic dispenser.
Microplates reader (450 nm, 620-630 nm)

Notes
Store all reagents between 2÷8°C in the dark.
Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, it is stable until expiry date of the kit.

4. WARNINGS
- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Calibrators and the Controls should be handled in the same manner as potentially infectious material.
- Some reagents contain small amounts of Sodium Azide (NaN_3) or Proclin 300^R as preservatives. Avoid the contact with skin or mucosa.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent 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/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.

5. PRECAUTIONS
- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as 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 date printed on box and vials labels must be observed. 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 not used properly;** therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, **for doses dispensed with the 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 the conjugate; this procedure is highly recommended when the kit is processed using analyzers which are not equipped with disposable tips.
  For this purpose, Dia.Metra supplies a separate decontamination reagent for cleaning needles.
- If you use automated equipment, the user has the 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. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition 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 the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.

- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of the Calibrators (C_0…C_4)

Since no international reference preparation for Anti DGP antibodies is available, the assay system is calibrated in relative arbitrary units. The Calibrators are ready to use and have the following concentration:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	15	30	60	240

Once opened, the Calibrators are stable 6 months at 2-8°C.

6.2. Preparation of the Sample

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; for example 10 L of sample may be diluted with 990 L 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 (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 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.

The Controls are ready to use.

6.3. Preparation of the 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. In concentrated wash solution is possible to observe the presence of crystals; in this case mix at room temperature until the complete dissolution of crystals; for greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care to transfer completely the crystals, then mix until crystals are completely dissolved.

6.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_4), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Controls	Blank
Calibrator C_0-C_4	**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 3 times with 300 µL of diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
Conjugate	**100 µL**	**100 µL**	
Incubate 30 minutes at room temperature (22-28°C). Remove the contents from each well, wash the wells 3 times with 300 µL of diluted wash solution. **Washing**: follow the same indications of the previous point.			
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 a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

7. RESULTS

7.1. Calibration curve

For Anti DGP IgA assay 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. 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 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 DGP IgA. These data are intended for illustration only and should not be used to calculate results from another run.

N	OD1	OD2	mean	C1	C2	mean	CV%
CAL0	0.013	0.009	0.011	0.18	0.00	0.09	141.42
CAL1	0.205	0.206	0.206	14.93	15.00	14.96	0.36
CAL2	0.401	0.405	0.403	29.89	30.20	30.04	0.73
CAL3	0.794	0.770	0.782	60.96	59.01	59.99	2.30
CAL4	2.558	2.710	2.634	231.2	249.0	240.1	5.26

8. REFERENCE VALUES

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti DGP tests:

	Anti DGP IgA (AU/mL)
Negative	< 15
Equivocal	15 - 30
Positive	> 30

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.
Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually.
It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti DGP.

9. PERFORMANCE AND CHARACTERISTICS

9.1. 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.

9.2. 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. Detection limit

The lowest concentration of Anti DGP IgA that can be distinguished from Calibrator 0 is less than 0.74 AU/mL with a confidence limit of 95%.

9.4. Precision and reproducibility

9.4.1. Intra-Assay

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

9.4.2. 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%.

10. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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Dia.Metra S.r.l.
Via Pozzuolo 14,
06038 SPELLO (PG) Italy
Tel. +39-0742-24851
Fax +39-0742-316197
E-mail: info@diametra.com

SUGGERIMENTI PER LA RISOLUZIONE DEI PROBLEMI/TROUBLESHOOTING

ERRORE CAUSE POSSIBILI/ SUGGERIMENTI

Nessuna reazione colorimetrica del saggio

- mancata dispensazione del coniugato
- contaminazione del coniugato e/o del Substrato
- errori nell'esecuzione del saggio (es. Dispensazione accidentale dei reagenti in sequenza errata o provenienti da flaconi sbagliati, etc.)

Reazione troppo blanda (OD troppo basse)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo breve, temperatura di incubazione troppa bassa

Reazione troppo intensa (OD troppo alte)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo lungo, temperatura di incubazione troppa alta
- qualità scadente dell'acqua usata per la soluzione di lavaggio (basso grado di deionizzazione,)
- lavaggi insufficienti (coniugato non completamente rimosso)

Valori inspiegabilmente fuori scala

- contaminazione di pipette, puntali o contenitori- lavaggi insufficienti (coniugato non completamente rimosso)

CV% intrasaggio elevato

- reagenti e/o strip non portate a temperatura ambiente prima dell'uso
- il lavatore per micropiastre non lava correttamente (suggerimento: pulire la testa del lavatore)

CV% intersaggio elevato

- condizioni di incubazione non costanti (tempo o temperatura)
- controlli e campioni non dispensati allo stesso tempo (con gli stessi intervalli) (controllare la sequenza di dispensazione)
- variabilità intrinseca degli operatori

ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run

- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)

- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

ERROR / POSIBLES CAUSAS / SUGERENCIAS

No se produce ninguna reacción colorimétrica del ensayo

- no se ha dispensado el conjugado
- contaminación del conjugado y/o del substrato
- errores en la ejecución del ensayo (p. ej., dispensación accidental de los reactivos en orden incorrecto o procedentes de frascos equivocados, etc.)

Reacción escasa (DO demasiado bajas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado corto, temperatura de incubación demasiado baja

Reacción demasiado intensa (DO demasiado altas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado largo, temperatura de incubación demasiado alta
- calidad escasa del agua usada para la solución de lavado (bajo grado de desionización)
- lavados insuficientes (el conjugado no se ha retirado completamente)

Valores inexplicablemente fuera de escala

- contaminación de pipetas, puntas o contenedores- lavados insuficientes (el conjugado no se ha retirado completamente)

CV% intraensayo elevado

- los reactivos y/o tiras no se encontraban a temperatura ambiente antes del uso
- el lavador de microplacas no funciona correctamente (sugerencia: limpiar el cabezal del lavador)

CV% interensayo elevado

- condiciones de incubación no constantes (tiempo o temperatura)
- controles y muestras no dispensados al mismo tiempo (con los mismos intervalos) (controlar la secuencia de dispensación)
- variación en función de los operadores

ERREUR CAUSES POSSIBLES / SUGGESTIONS

Aucune réaction colorimétrique de l'essai

- non distribution du conjugué
- contamination du conjugué et/ou du substrat
- erreurs dans l'exécution du dosage (par ex., distribution accidentelle des réactifs dans le mauvais ordre ou en provenance des mauvais flacons, etc.)

Réaction trop faible (DO trop basse)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop court, température d'incubation trop basse

Réaction trop intense (DO trop élevée)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop long, température d'incubation trop élevée
- mauvaise qualité de l'eau utilisée pour la solution de lavage (bas degré de déionisation)
- lavages insuffisants (conjugué non entièrement éliminé)

Valeurs inexplicablement hors plage

- contamination des pipettes, embouts ou récipients - lavages insuffisants (conjugué non entièrement éliminé)

CV% intra-essai élevé

- les réactifs et/ou les bandes n'ont pas atteint la température ambiante avant usage
- le laveur de microplaques ne lave pas correctement (suggestion : nettoyer la tête du laveur)

CV% inter-essai élevé

- conditions d'incubation non constantes (temps ou température)
- contrôles et échantillons non distribués en même temps (avec les mêmes intervalles) (contrôler l'ordre de distribution)
- variabilité intrinsèque des opérateurs