Product information

Instruction for use

DGP Ab IgG ELISA

Enzyme Immunoassay for the quantitative detection of IgG-antibodies against deamidated gliadin protein epitopes (DGP) in human serum or plasma

CE

IVD

REF **DE7780**

Σ **96 Tests**

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JS
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PRINCIPLE OF THE TEST

Deamidated gliadin proteins are bound to microwells.
The determination is based on an indirect enzyme linked immune reaction with the following steps:
Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subesquently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stopps the reaction generating a yellow end-product. The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

SUMMARY AND EXPLANATION OF THE TEST

Celiac disease (CD) comprises intolerance against dietary gluten present in wheat, rye and barley, and it belongs to the most common food-related diseases. Nowadays, CD is conceived as an autoimmune-mediated systemic disorder commonly presenting as enteropathy in genetically susceptible individuals.

Four possible presentations of CD have been recognized:
1. Typical, characterized mostly by gastrointestinal signs and symptoms.
2. Atypical or extra intestinal, where gastrointestinal symptoms are minimal or absent and a number of other manifestations are present.
3. Silent, where the small intestinal mucosa is damaged and CD autoimmunity can be detected by serology, but there are no symptoms.
4. Latent, where individuals possess genetic compatibility with CD and may also show positive autoimmune serology, but have normal mucosa morphology and may or may not be symptomatic.

The most obvious feature distinguishing CD from other small-intestinal enteropathies is the presence of autoantibodies against the key autoantigen tissue transglutaminase (abbreviated as TG2 or tTG) during a gluten containing diet. The gluten-derived gliadin peptides and the self antigen tTG, play a role in CD pathogenesis. Determination of serum levels of immunoglobulin A (IgA) against tTG is the first choice in suspected CD, displaying the highest levels of sensitivity and specificity. tTG is known to deamidate and crosslink gluten-derived gliadin peptides between a lysine and a glutamine residue. The interplay between gliadin peptides and tTG is responsible for the generation of novel antigenic epitopes, the tTG-generated deamidated gliadin peptides (DGP). Such peptides represent much more CD-specific epitopes than native gliadin peptides, and anti-DGP antibodies are promising serological markers for CD. Endomysial antibodies (EMA) complement the repertoire of CD specific antibodies. EMA testing with indirect immunofluorescence methods may be a useful alternative if the result of the tTG test is equivocal. Tests for the detection of IgG or IgA antibodies against native gliadin peptides may be valuable to document adherence to the gluten-free diet administered by the treating physician – the very effective but until now also the only possible treatment of CD.
Laboratory tests for disease specific autoantibodies contribute to diagnostics, together with clinical observations and histology of the small intestinal mucosa: The characteristic celiac lesions with villous atrophy, crypt hyperplasia, and increased intraepithelial lymphocytosis in duodenal biopsy samples.
In 2012 a working group of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) published new Guidelines for the diagnosis of celiac disease in children and adolescents. Major changes where made concerning the claim for duodenal biopsy. They defined subsets of patients for whom biopsies where avoidable. As CD may present with a large variety of nonspecific symptoms, it is important to examine not only those patients with obvious gastrointestinal troubles but also persons with a less clear clinical picture and to distinguish between symptomatic and asymptomatic patients.

Symptomatic patients:
The initial test should be IgA anti-tTG from a blood sample. IgA anti-DGP may be used as additional test in patients who are negative for other CD-specific antibodies but in whom clinical symptoms raise a strong suspicion of CD, especially if they are younger than two years. In subjects with either primary or secondary humoral IgA deficiency, at least one additional test measuring IgG class CD-specific antibodies is recommended (IgG anti-tTG, IgG anti- DGP, IgG EMA, or blended kits for both IgA and IgG antibodies). The clinical relevance of a positive anti-tTG or anti-DGP result should be confirmed by histology, unless certain conditions are fulfilled that allow the option of omitting the confirmatory biopsies.

Requirements for diagnosing CD without duodenal biopsy:
In children and adolescents with symptoms suggestive of CD and high anti-tTG titers (levels >10 times upper limit of normal) the paediatric gastroenterologist may discuss the option of performing further laboratory testing (EMA, HLA-typing) to make the diagnosis of CD without biopsies.

Asymptomatic persons at risk for CD:
In individuals without clinical signs and symptoms but with an increased genetic risk for CD, an anti-tTG IgA test and total IgA determination should be performed, preferably not before the child is two years old. If antibodies are negative, then repeated testing for CD-specific antibodies on a gluten containing diet is recommended. Duodenal biopsies demonstrating the characteristic celiac lesions should always confirm the results of serologic tests.

Follow-up
If the diagnosis is definitely made the patient can start a gluten free diet. Patients should be followed up regularly for symptomatic improvement and normalisation of CD-specific antibody tests. About twelve months after onset of the gluten free diet antibody titres usually decrease below the detection limit.

CONTENTS OF THE KIT

Sufficient for 96 determinations

1 One divisible microplate consisting of 12 modules of 8 wells each. Ready to use.

6x 1.5 ml Calibrator A-F (0, 6.3, 12.5, 25, 50, 100 U/ml), containing DGP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.

2x 1.5 ml Control positive (1) and negative (2), containing DGP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.

20 ml Sample Buffer P, containing PBS, BSA, detergent, preservative NaN_3 0.09%, yellow. 5x conc.

15 ml Enzyme Conjugate light red, containing anti-human IgG antibodies, HRP labelled; PBS,

BSA, detergent, preservative ProClin 300 0.05%. Ready to use.

15 ml TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.

15 ml Stop Solution; contains acid. Ready to use.

20 ml Wash Solution, containing Tris, detergent, preservative NaN_3 0.09%; 50 x conc.

1 Instruction for Use

1 Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and dessicated in the clip bag provided.
- Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C.
- We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, performe the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
- Control, calibrator, sample buffer and wash solution contain sodium azide (NaN_3) 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as
- non-hazardous.
- During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:
- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:
- Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
- Exposure controls / personal protection: Wear protective gloves of nitrile rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.
- Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

PREPARATION OF REAGENTS

Wash Solution

Dilute the contents of one vial of the buffered wash solution concentrate (50 x) with distilled or deionised water to a final volume of 1000 ml prior to use.

Sample Buffer

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well.

Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette 100 µl of calibrators, controls and prediluted patient samples into the wells.
2. Incubate for 30 minutes at room temperature (20-28 °C).
3. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
4. Dispense 100 µl of enzyme conjugate into each well.
5. Incubate for 15 minutes at room temperature.
6. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
7. Dispense 100 µl of TMB substrate solution into each well.
8. Incubate for 15 minutes at room temperature
9. Add 100 µl of stop solution to each well of the modules
10. Incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm (reference 600-690 nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

P1, ... patient sample A-F calibrators C+, C- controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit. If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.
Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

Calibration
This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

Measuring range
The calculation range of this ELISA assay is 0 – 100 U/ml.

Expected values
In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 10 U/ml

Interpretation of results
Negative: < 10 U/ml
Positive: ≥ 10 U/ml

Linearity
Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	55.3	55.3	100
.	1:200	27.0	27.7	97
.	1:400	13.9	13.8	101
.	1:800	7.3	6.9	106
.	1:1600	3.4	3.5	97
2	1:100	69.1	69.1	100
.	1:200	37.8	34.6	109
.	1:400	20.2	17.3	117
.	1:800	9.8	8.6	114
.	1:1600	4.3	4.3	100

Limit of detection
Functional sensitivity was determined to be: 1 U/ml

Interfering substances
No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparin). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Reproducibility
Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample .	Mean U/ml	. CV %
1	80.3	4.6
2	37.0	4.3
3	17.2	5.8

Inter-Assay		
Sample .	Mean U/ml	. CV %
1	88.0	2.7
2	59.8	2.5
3	17.8	4.1

Study results

Study population	n	n Pos	%
Coeliac disease	40	37	92.5
Normal human sera	50	0	0.0

Clinical Diagnosis

	Pos	Neg	
Pos	37	0	
Neg	3	50	
	40	50	90

Sensitivity: 92.5 %
Specificity: 100.0 %
Overall agreement: 96.7 %

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually. The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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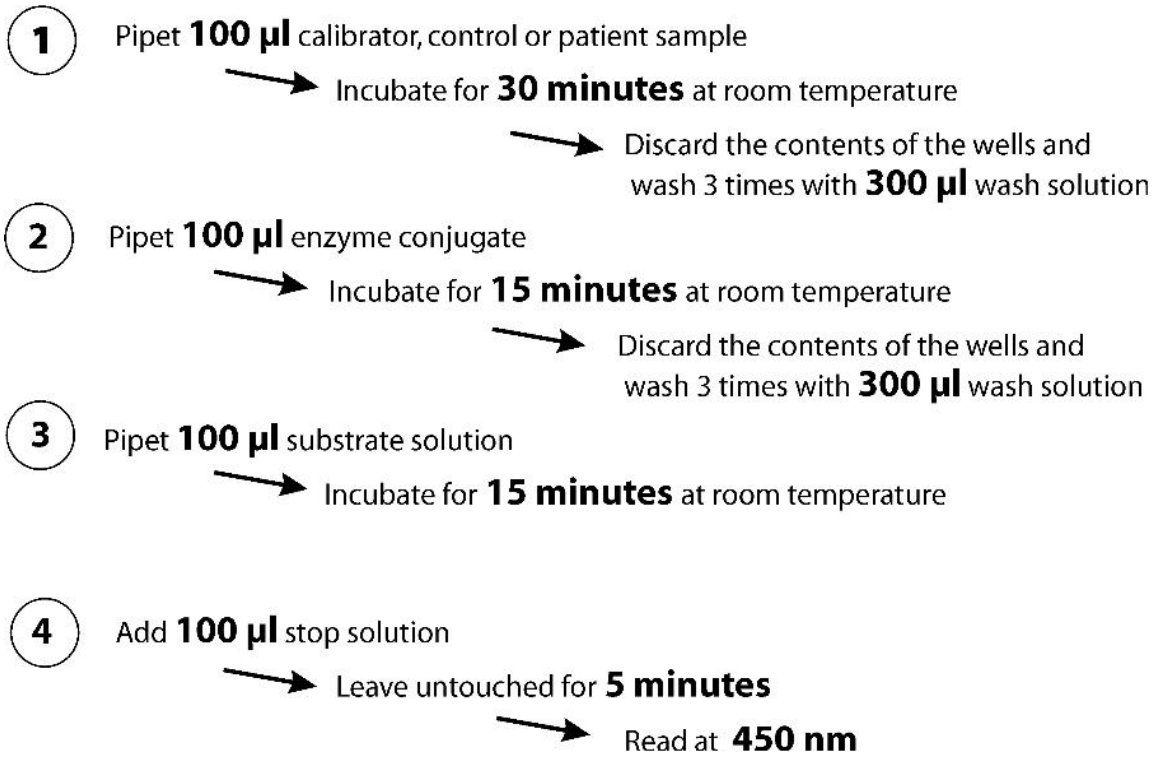
1
Pipet 100 µl calibrator, control or patient sample
Incubate for 30 minutes at room temperature
Discard the contents of the wells and wash 3 times with 300 µl wash solution
2
Pipet 100 µl enzyme conjugate
Incubate for 15 minutes at room temperature
Discard the contents of the wells and wash 3 times with 300 µl wash solution
3
Pipet 100 µl substrate solution
Incubate for 15 minutes at room temperature
4
Add 100 µl stop solution
Leave untouched for 5 minutes
Read at 450 nm

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

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