DIAWell Deamidated Gliadin IgG Elisa

KAPDTDGLG02

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DIAsource ImmunoAssays S.A. - Rue du Bosquet, 2 - B-1348 Louvain-la-Neuve - Belgium

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96 test Enzyme Immunoassay

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RESEARCH USE ONLY

DIAsource ImmunoAssays SA - Rue du Bosquet 2, B-1348 Louvain-la-Neuve, Belgium - Tel: +32 10 84 99 11 - Fax : +32 10 84 99 90

INTENDED USE

The DIAWell Deamidated Gliadin IgG ELISA kit allows the detection of IgG antibodies to deamidated gliadin in human serum.

PRINCIPLE OF THE TEST

The DIAWell Deamidated Gliadin IgG is a solid phase enzyme immunoassay using 96 coated breakaway microwells and a peroxidase-TMB detection system. The microwells are coated with highly specific antigen.
In the test procedure, serum samples are diluted 1/51 and incubated in the microwells. Human antibodies, if present, bind to the specific antigen. Unbound or excess antibodies are removed by washing and HRP-conjugated rabbit antibodies against human IgG are added to the microwells. The enzyme conjugate binds to the antigen-antibody complexes. After a second washing step to remove excess conjugate, the TMB/substrate solution is added. The enzyme activity, if present, generates a colorimetric (blue) reaction. Diluted acid is added to stop the reaction. Consequently the colour turns from blue to yellow and may be measured at 450 nm using a conventional microplate reader. The absorbance (Optical Density) is directly proportional to the concentration of IgG antibodies bound to the antigen on the microwells surface.

KIT CONTENTS : MATERIAL PROVIDED IN THE KIT

TO BE RECONSTITUED :

WASH	SOLN	CONC	**(20 x) Wash buffer**	**1 vial, 50 ml – 20 x concentrated (blue)** *Containing : Tris, Tween, Methylisothiazolone (Preservative)*

READY TO USE :

DIL	SPE	**Sample Diluent**	**1 vial, 50 ml (yellow)** *Containing : Tris, Tween, BSA, Methylisothiazolone (preservative)*
SUB		**Substrate**	**1 vial, 20 ml (colourless)** *Containing : stabilized TMB/H_2O_2, Methylisothiazolone (preservative)*
Control	L	**Negative Control**	**1 vial, 1 ml (green)** *Containing : human serum (diluted), Methylisothiazolone (preservative)*
Cal	N	**Calibrator**	**6 vials, 1 ml each 0, 25, 50, 100, 200, 400 U/ml (colour increasing with concentration)** *Containing : human serum (diluted), Methylisothiazolone (preservative)*
Control	H	**Positive Control**	**1 vial, 1 ml (blue)** *Containing : human serum (diluted), Methylisothiazolone (preservative)*
Ab	HRP	**HRP Conjugate**	**1 vial, 20 ml (red)** *Containing : Rabbit anti-human IgG/peroxidase, Methylisothiazolone (preservative)*
STOP	SOLN	**Stop solution**	**1 vial, 20 ml (colourless)** *Containing : sulphuric acid 2.5 %*
		Microtiterplate	**12 x 8 well strips with breakaway microwells** *Coated with purified deamidated gliadin*

Frame for strips **1**

MATERIAL REQUIRED BUT NOT PROVIDED

- Microtiter plate reader (450 nm reading filter + optional 650 nm reference filter).
- Glass ware, test tubes for the dilutions.
- Distilled water.
- Precision pipettes (10, 100, 200, 500, 1000 µl) or multipipette.
- Microplate washing device (multichannel pipette or automated system)
- Absorbent paper.

STORAGE

- Store all reagents and microwells at 2-8°C
- Once prepared the washing solution is stable for 1 month at 4°C.
- Reagents and microwells should be used until the expiry date indicated on each component only.

PRECAUTIONS

1. Health hazard data

THIS PRODUCT IS FOR RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following recommendations and precautions for maximum safety when handling:

- The kit contains potentially hazardous components. Reagents may be irritating to eyes and skin thus avoid contact with eyes and skin. Do not smoke, eat or drink when manipulating the kit.
- All human source material used for some reagents of this kit (controls, calibrators) has been tested and found negative for HbsAg, for Hepatitis C and for HIV 1 and 2 antibodies by approved methods. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, calibrators and samples as if capable of transmitting infectious diseases.

2. Other precautions

- Do not mix or substitute reagents or microwells from different lot numbers. This may lead to variations in the results.
- Allow all components to reach room temperature (18-24C) before use and follow the recommended incubation scheme for an optimimum performance of the test
- Always pipette reagents with clean tips in order to avoid contamination with exogenous substances.

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- Protect the chromogen / substrate reagent from light to avoid increase in blank values.

SAMPLE COLLECTION, HANDLING AND STORAGE

- Use preferentially freshly collected serum samples.
- Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be clarified by low speed centrifugation.
- Blood samples should be collected in dry tubes. After separation, the serum samples should be used immediately, respectively stored at 2-8°C for two or three days, or frozen at -20°C for longer periods.

ASSAY PROCEDURE

1. Samples

- Dilute serum samples **1:51** with sample diluent (ready-to-use)
  → **e.g. 500 µl** diluent + **10 µl** serum. **Mix.**

2. Wash buffer

- Dilute the concentrated Wash buffer **1:20** with distilled water

◊ ***Manual washing:*** Prepare **10 ml** final volume per **8 wells** or **120ml** for **96 wells**
→ **e.g. 9.5 ml** water + **0.5 ml** buffer. Mix.

◊ ***Automated washing:*** consider excess volumes required for setting up the instrument and dead volume of robot pipette.

3. Microwells

- Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store them in the provided plastic bag, sealed tightly

4. Pipetting Scheme

- Make sure all reagents are at room temperature before use (18-24°C)

- **Pipette 100 µl** of each **diluted serum** into the designated microwells.
- **Pipette 100 µl calibrators and controls** into the designated wells.
- **Incubate** for **30 minutes** at room temperature (18-24°C).
- **Wash 3 X** with **200 µl washing buffer (diluted 1:20)**.
- **Pipette 100 µl conjugate** into each well.
- **Incubate** for **30 minutes** at room temperature (18-24°C).
- **Wash 3 X** with **200 µl washing buffer (diluted 1:20)**.
- **Pipette 100 µl substrate** into each well.
- **Incubate** for **10 minutes** at room temperature (18-24°C).
- **Pipette 100 µl stop solution** into each well, using the same order as pipetting the substrate.
- **Read absorbance** at **450 nm** (optionally 450/650 nm) within 30 minutes.

NOTE: We recommend to pipette a blank in duplex with each run (sample diluent only, instead of a sample).

Manual washing procedure
Discard liquid from wells by inverting the plate. Knock the microwell frame with wells down-sided vigorously on clean absorbent paper. Pipette 200 µl of diluted wash buffer into each well, wait for 20 seconds, repeat discard and knocking. Repeat the whole procedure twice again.

CALCULATION AND INTERPRETATION OF THE RESULTS

1. Quantitative interpretation

Establish the calibration curve by plotting the optical density of each calibrator with respect to the corresponding units values. For best results we recommend lin/lin algorithm. From the O.D. of each sample, read the corresponding antibody concentrations expressed in U/ml.

Normal Range: IgG ≤ 50 U/ml

INTERPRETATION	**Negative result**	**Positive result**
	≤ 50 U/ml	**> 50 U/ml**

NOTE: Borderline samples should be tested again for confirmation.

2. Semi-quantitative interpretation
A semi-quantitative interpretation of the results is available by using the **50 U/ml** calibrator as a cut off control. Results are expressed in **B**inding **I**ndex, the ratio between the sample and the cut off's O.D.:

B.I. = Sample O.D / Cut-off O.D

A sample is **negative** when **B.I. ≤ 1.0**
A sample is **positive** when **B.I. > 1.0**

NOTE: Borderline samples should be tested again for confirmation.

3. Validation of results
A test run is considered valid if the following Quality Control specifications are met.
If not check the whole procedure and repeat the test. If the problem persists call manufacturer or distributor for assistance.

	Quality Control specifications	
	O.D.	**U/ml**
Blank (sample diluent)	< 0.100	-
Negative control	< 20% Positive Control	≤ 40
50 U/ml Calibrator	< 50 % of Calibrator 400 U/ml	-
Positive control	> 0.800	200 – 400

PERFORMANCES

1. **Linearity**
Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies individual samples may not follow this rule in every case. Detailed and updated data are available upon request.

2. **Reproducibility**
Three control sera (High, medium, low) were assayed for intraassay and interassay imprecision in a statistically relevant repetition. The variation coefficients are <10% intra- and <20% inter-lot. Detailed and updated data are available upon request.

3. **Sensitivity and Specificity**

Sensitivity is estimated to be 70.6 %
Specificity is estimated to be 95.5 %
Defined populations (confirmed positive with disease specific reference methodologies) have been used for checking the sensitivity. Specificity was checked with control groups that embrace a normal healthy population as well as defined control groups. Detailed data are available upon request.

4. Expected Values
The expected value for a normal serum is a negative result. The number of positives, and the degree of positivity is dependent upon parameters such as population type being tested, treatment, etc. Each laboratory should consequently establish its own expected values based upon the specimens typically being tested.

TEST LIMITATIONS

1. All test results shoudl be use for reserach use only purposes.
2. DIAsource ImmunoAssays SA and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
3. In any case, GLP should be applied with all general and individual regulations to the use of this kit.

More particularly, it must be emphasized that anti-deamidated gliadin IgG antibodies are of limited value for the determination of Celiac disease, due to low specificity. Therefore, anti-deamidated gliadin IgG antibodies should be investigated and results interpreted with care, in established IgA-deficient subjects only.

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BASIC TROUBLE SHOOTING

Optical density too low	Optical density too high
Please, check the following possibilities: ● Inappropriate reader filter (use 450 nm or 450/650nm) ● Correct dilution of washing buffer (under-diluted) ● Correct dilution of samples (over-diluted) ● Inactivation of conjugate (by exogenous substances e.g.). Use clean tips only.	Please, check the following possibilities: ● Insufficient washing (See manual washing procedure) ● Excess incubation time or temperature ● Correct dilution of samples (under-diluted) ● Contamination of substrate reagent (by conjugate e.g. → color obviously blue already in the bottle). Use clean tips only. ● Contamination of samples (by micro-organisms e.g.). Use preferentially fresh samples.

BIBLIOGRAPHY

Up to date literature is available upon request. Please inquire at Tech.Support@diasource.be.

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MANUFACTURED FOR:
Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave NE, Ste P
Minneapolis, MN 55432
Tel: 763-780-2955
Fax: 763-780-2988
Email: info@ibl-america.com
Web: www.ibl-america.com

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	Used symbols
	Consult instructions for use
	Storage temperature
	Use by
LOT	Batch code
REF	Catalogue number
CONTROL	Control
IVD	In vitro diagnostic medical device
	Manufacturer
Σ	Contains sufficient for <n> tests
WASH SOLN CONC	Wash solution concentrated
CAL 0	Zero calibrator
CAL N	Calibrator #
CONTROL N	Control #
Ag 125I	Tracer
Ab 125I	Tracer
Ag 125I CONC	Tracer concentrated
Ab 125I CONC	Tracer concentrated
	Tubes
INC BUF	Incubation buffer
ACETONITRILE	Acetonitrile
SERUM	Serum
DIL SPE	Specimen diluent
DIL BUF	Dilution buffer
ANTISERUM	Antiserum
IMMUNOADSORBENT	Immunoadsorbent
DIL CAL	Calibrator diluent
REC SOLN	Reconstitution solution
PEG	Polyethylene glycol
EXTR SOLN	Extraction solution
ELU SOLN	Elution solution
GEL	Bond Elut Silica cartridges
PRE SOLN	Pre-treatment solution
NEUTR SOLN	Neutralization solution
TRACEUR BUF	Tracer buffer
	Microtiterplate
Ab HRP	HRP Conjugate
Ag HRP	HRP Conjugate
Ab HRP CONC	HRP Conjugate concentrate
Ag HRP CONC	HRP Conjugate concentrate
CONJ BUF	Conjugate buffer
CHROM TMB CONC	Chromogenic TMB concentrate
CHROM TMB	Chromogenic TMB solution
SUB BUF	Substrate buffer
STOP SOLN	Stop solution
INC SER	Incubation serum
BUF	Buffer
Ab AP	AP Conjugate
SUB PNPP	Substrate PNPP
BIOT CONJ CONC	Biotin conjugate concentrate
AVID HRP CONC	Avidine HRP concentrate
ASS BUF	Assay buffer
Ab BIOT	Biotin conjugate
Ab	Specific Antibody
SAV HRP CONC	Streptavidin HRP concentrate
NSB	Non-specific binding
2nd Ab	2nd Antibody
ACID BUF	Acidification Buffer
DIST	Distributor
TRAY	Incubation trays
PMSF	PMSF solution
	Protect from light
STRIP	Dot Strip
SUB	Substrate
EXTR SOLN CONC	Extraction Buffer Concentrate
CART	Cartridge
SAV HRP	Streptavidin HRP
PIPETTE	Pipette
WASH SOLN	Wash buffer

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