EPITOPE DIAGNOSTICS, INC.

EDI™ Deamidated Gliadin Peptide, IgA CLIA Kit

Chemiluminescence Immunoassay (CLIA) for the quantitative measurement of DGP-IgA in Serum.

REF CL0007R RUO 50, 100, 250 +2 +8 °C

INTENDED USE

This Chemiluminescence Immunoassay (CLIA) kit is intended for the quantitative determination of human Deamidated Gliadin Peptide, IgA (DGP-IgA) levels in serum using the ECL100 or ECL25 Immunoassay analyzer. The test is used as an aid in the diagnosis of celiac disease.

For Research Use only

SUMMARY OF PHYSIOLOGY

In celiac disease, when the proteins found in gluten are detected, the body produces antibodies as an immune response to the perceived threat. In this process, the immune system causes damage to the villi in the small intestine, which can lead to many symptoms including abdominal pain, malnutrition, and fatigue[1]. One of these antibodies produced as a reaction to the presence of gluten is DGP-IgA, or deamidated gliadin peptide, IgA.

Typically, in the celiac disease diagnostic process, the first serological test recommended is for tissue transglutaminase, or tTG[2]. While tTG tests show high sensitivity and specificity in most cases, they are not always conclusive. When tTG test results cannot conclusively diagnose celiac disease or rule out a diagnosis, deamidated gliadin peptide test can be recommended[3].

ASSAY PRINCIPLE

This CLIA is designed, developed, and produced for the quantitative measurement of human DGP-IgA in serumsamples. The assay utilizes a two-site "sandwich" technique with one antigen and two antibodies that bind to different epitope and paratope of DGP-IgA.

Assay calibrators, controls, or patient samples are added directly to a reaction vessel containing streptavidin coated magnetic particles. Simultaneously, anacridinium ester antibody and a biotin antigenare added. The magnetic particles capture the biotin antigen as well as an immuno complex in the form of "magnetic particles – biotin DGP-IgA antigen –DGP-IgA– acridinium ester DGP-IgA antibody".

The materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the trigger solution is added to the reaction vessel and light generated by the reaction is measured with the ECL100 or ECL25 analyzer. The relative light units (RLU) are proportional to the concentration of DGP-IgA in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve and reported in serum DGP-IgA concentration.

REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date. Reagents from different kit lot numbers should not be combined or interchanged.

Standard Batch Quantity: 100/kit

1. DGP-IgA Magnetic Particle Solution (L0663)
Qty: 1 x 2.0 mL (50/kit), 1 x 2.8 mL (100/kit), 1 x 7.3 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

2. Biotin DGP-IgA Antigen (L0664)
Qty: 1 x 3.5 mL (50/kit),1 x 6.0 mL (100/kit), 1 x13.5 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

3. Acridinium Ester DGP-IgA Antibody (L0665)
Qty: 1 x3.5 mL (50/kit), 1 x6.0 mL (100/kit), 1 x 13.5 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

4. DGP Dilution Buffer (L0677)
Qty: 1 x10.5 mL (50/kit), 1 x 20.0 mL (100/kit), 1 x 48.5 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

5. DGP-IgA Calibrators (L0666 – L0667)
Liquid human DGP-IgA in a PBS-based matrix with an azide preservative. Refer to vials for exact concentration.
Qty: 2 x vials of 0.5 mL each
Storage: 2 – 8°C
Preparation: 0.5 mL of Calibrators, mix by inversions or gentle vortexing. Make sure that Calibrators are well mixed before use.

6. DGP-IgA Controls (L0668 – L0669)
Liquid human DGP-IgA in a PBS-based matrix with an azide preservative. Refer to vials for exact concentration.
Qty: 2 x vials of 0.5 mL each
Storage: 2 – 8°C
Preparation: 0.5 mL of Controls, mix by inversions or gentle vortexing. Make sure that Controls are well mixed before use.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for in vitro diagnostic use. Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide. Do not get in eyes, on skin, or on clothing. Do not ingest

or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

The instrument only uses materials supplied by Epitope Diagnostics, Inc. When materials available from third-party suppliers are used, Epitope Diagnostics, Inc. takes no responsibility for the validity of results obtained. Material is available for purchase from Epitope Diagnostics, Inc. Please contact your distributor for more information.

1. ECL100 Immunoassay Analyzer or ECL25 Immunoassay Analyzer
2. CL011 Cuvettes (for ECL100) or CL010 Cuvettes (for ECL25)
3. Wash Reagent (P-594)
4. Trigger Solutions A and B (P-595)

SPECIMEN COLLECTION AND PREPARATION

Only 10 µL of human serum or plasma sample is required for DGP-IgA measurement in singlet. Samples should not be taken from patients taking biotin-containing multivitamins or dietary supplements at least 48 hours prior to specimen collection. Whole blood should be collected and must be allowed to clot for minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500 RPM for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube. Serum samples should be stored at 15-25°C for three days, 2-8°C for five days, and –20°C or below for three months. Avoid more than three freeze-thaw cycles of specimen. It is necessary to take care in the sample collection procedure to avoid hemolysis.

Some substances in the samples will interfere with the test results. The common interfering substances and maximum allowable concentrations are as follows:

- bilirubin 60 mg/dL
- triglycerides 1500 mg/dL
- hemoglobin 900 mg/dL
- biotin 200 nmol/L
- For patients receiving high-dose biotin therapy (5 mg/ day), samples must be collected 8 hours after takingthe last dose of biotin

A single assay of this item requires 10 µL sample. This quantity does not include the deadvolume in the sample container, the capacity required for retesting, and other measurement items. For the definition of the minimum required sample size, refer to the equipment manual.

CALIBRATION

An active calibration curve is required for all tests. For the assay, calibration must be performed when a reagent lot is used for the first time and remains valid for 28 days. After this period, recalibration is required. Additionally, we recommend performing calibration if control results fall outside the acceptable range.

QUALITY CONTROL

The use of controls is left to the discretion of the user, based on good laboratory practices, requirements, and applicable laws. It is strongly recommended to perform a control test before running patient samples. If no patient samples are tested, a control test is not necessary. Quality control results that fall outside the acceptable range may indicate invalid test results. Please refer to the Certificate of Analysis for the correct control range.

ASSAY PROCEDURE

1. Reagents from different kit lot numbers should not be combined or interchanged. Make sure that there are no air bubbles in any reagents, calibrator, and control vials.

2. Reagent Preparation

2.1 Remove reagent cartridges from packaging and replace the solid caps with the provided soft caps for ECL100. For ECL25, carefully remove the aluminum foil seal on each container on the cartridges.

2.2 For the ECL100, take out the Magnetic Particle bottle and make sure to roll between hands and gently but thoroughly mix until the magnetic particle solution is homogenous. The solution should be uniform with no clumps of magnetic particles visible; this step is vital for assay performance.

- Note: For ECL 100, if the Magnetic Particle Solution volume is over 2.8 mL, it will be supplied in a glass bottle. It will need to be transferred from the glass bottle to the plastic vial in the cartridge with the rest of the reagents. Please note: The maximum transfer volume of the Magnetic Particle Solution is 2.8 mL. Make sure the Magnetic Particle Solution is mixed thoroughly before transferring.

2.3 For ECL25, mix the magnetic beads by moving back and forth the bottom part of the cartridge at upright position. Make sure to look inside the cartridge until the solution is uniform with no clumps of magnetic particles visible and no air bubbles. Recap the bottle. Open the top soft cap of all reagent bottles, leaving only the hollow soft rubber.

2.4 The reagents are now ready to be loaded into the ECL100 or ECL25 for calibration.

3. Assay Program

The following table illustrates the protocol used by the ECL100 or ECL25 for instrument operation.

Component	Quality Control Hole (µL)	Sample Hole (µL)
DGP-IgA Calibrators (L0666-L0667)	10	-
Samples	-	10
DGP DilutionBuffer (L0677)	190	190
Biotin DGP-IgA Antigen (L0664)	50	50
DGP-IgA Magnetic Particle Solution (L0663)	25	25
Incubation Period 1		
Wash the reaction cup 3 times with the wash reagent.		
Acridinium Ester DGP-IgA Antibody (L0665)	50	50
Incubation Period 2		
Wash the reaction cuvette 3 times with wash reagent.		
Trigger Solution A (P-595)	200	200
Trigger Solution B (P-595)	200	200

The total incubation time is less than 30 minutes.

INTERPRETATION OF RESULTS

The chemiluminescence analyzer calculates the concentration values of the sample and the control by a standard curve (fitting method: four parameters or point-to-point) and the measured RLU. Values are compared with the range of the marked value. If it exceeds the indicated quality control range, it indicates that the test is unqualified and needs to be re-tested.

Due to methodological differences or antibody/antigen specificity, there may be deviations between the test results of

reagents from different manufacturers. Therefore, direct comparisons should not be made to avoid false interpretation.

EXPECTED VALUES

DGP-IgA concentrations were measured in serum samples collected from 125 apparently healthy adults using the EDI™ Deamidated Gliadin Peptide, IgA CLIA Kit. The observed range of DGP-IgA is summarized in the table below.

	DGP-IgA Concentration
Normal	0.00 – 14.00U/mL
Elevated	> 14.00 U/mL

It is highly recommended that each laboratory should establish their own normal range for DGP-IgA based on local populations.

LIMITATIONS OF THE PROCEDURE

1. This product is for use on the ECL100 or ECL25Immunoanalyzer only. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, operation, system performance, instructions, calibration, precautions, hazards, maintenance, and troubleshooting.
2. Reagents from different lots cannot be mixed.
3. Test results from this product should not be the sole basis for clinical diagnosis.
4. If the test sample result is higher than the upper limit of the calibration curve, it is recommended to re-measure after dilution according to a certain ratio. The measurement result is recalculated according to the dilution ratio to ensure the accuracy of the result.
5. When the sample concentration of DGP-IgA is lower than the detection lower limit, the test result will be reported as <0.40 U/mL. When the sample concentration is higher than the detection upper limit, it will be reported as >140.00 U/mL.

PERFORMANCE CHARACTERISTICS

Hook Effect

The assay shows no hook effect up to 2800.00U/mL.

Limit of Blank

The limit of blank (LoB) was determined by 60 replicates in three assays of calibrator matrix to be 0.13U/mL.

Limit of Detection

The limit of detection (LoD) was determined by 60 replicates in three assays of low-level samples to be 0.40U/mL.

Limit of Quantification

The limit of quantification (LoQ) was determined by 60 replicates in three assays of low-level samples to be 0.67U/mL.

Linearity

Linearity was determined by two assays with a diluted standard of high DGP-IgA concentration. In each assay, the average of two replicates of each of the diluted samples is used for a correlation analysis against calculated theoretical values. The linearity of this test is up to 140.00U/mL.

Standard	Average Concentration (U/mL)	Theoretical Concentration (U/mL)	Linear Recovery (%)	R^2
1	0.00	0.00	-	0.997
2	2.20	2.19	101	
3	4.18	4.38	96	
4	8.49	8.75	97	
5	16.74	17.50	96	
6	32.37	35.00	92	
7	69.27	70.00	99	
8	147.68	140.00	105	

Intra-assay Precision

Precision was determined by measuring eight replicates of three specimens. The results are as follows:

Sample	Average Concentration (U/mL)	SD	CV (%)
1	3.78	0.22	5.8
2	14.49	0.44	3.0
3	57.73	1.82	3.2

Inter-assay Reproducibility

Reproducibility was determined by measuring three specimens in twenty-four replicates over the run of three assays. The results are summarized below:

Sample	Average Concentration (U/mL)	SD	CV (%)
1	3.74	0.24	6.4
2	14.39	0.50	3.5
3	58.60	2.54	4.3

Cross Reactivity

Cross-reactivity was assessed by analyzing several specimens containing several analytes at elevated concentrations. The results are summarized below:

Analytes	Theoretical Concentration	Measure Concentration (U/mL)
Anti-TPO Antibody	37.33 IU/mL	< 0.100
Anti-TG Antibody	1047.30 IU/mL	< 0.100
TG Antibody	514.13 ng/mL	< 0.100
Human Ferritin	2240.40 ng/mL	< 0.100
Human Squamous cell Carcinoma Antigen	71.46 mg/mL	< 0.100

Interference

Bilirubin, hemoglobin, and Intralipid were tested as potential interferents to DGP-IgA. Randomly selected samples were spiked with the potential interferents at the concentrations listed in the table below:

Interferent (Concentration tested)		Test (U/mL)	Control (U/mL)	Bias (d_{obs}, %)
Bilirubin	0.005 mg/mL	4.20	4.40	-4.4
		18.05	17.42	3.6
	0.01 mg/mL	4.07	4.40	-7.5
		18.44	17.42	5.8
	0.02 mg/mL	4.15	4.40	-5.6
		17.63	17.42	1.2
Hemoglobin	0.05 mg/mL	4.10	4.22	-2.8
		18.03	17.06	5.7
	1.0 mg/mL	4.11	4.22	-2.5
		18.62	17.06	9.2
	2.0 mg/mL	4.27	4.22	1.2
		18.29	17.06	7.2
Intralipid	1.0 mg/mL	4.25	4.10	3.7
		18.25	17.85	2.3
	5.0 mg/mL	4.25	4.10	3.7
		18.80	17.85	5.3
	10.0 mg/mL	3.97	4.10	-3.2
		17.78	17.85	-0.4

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the

purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCES

1. Harvard Health. Celiac disease. Harvard Health. Published July 18, 2023. https://www.health.harvard.edu/diseases-and-conditions/celiac-disease#:~:text=When%20people%20with%20celiac%20disease%20eat%20foods%20containing,fingerlike%20projections%20in%20the%20small%20intestine%20called%20villi.
2. Seehusen DA. Comparative accuracy of diagnostic tests for Celiac disease. AAFP. Published June 1, 2017. https://www.aafp.org/pubs/afp/issues/2017/0601/p726.html
3. Celiac disease screening | Celiac Disease Foundation. Celiac Disease Foundation. https://celiac.org/about-celiac-disease/screening-and-diagnosis/screening/

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

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Please visit our website at www.epitopediagnostics.com to learn more about our products and services.

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)