

**CONTENTS**

EliA uses a modular reagent system. All information needed to understand the use of the EliA tests can be found in the analyte specific DfU and the corresponding EliA Control DfU on this CD.

**INTENDED USE**

EliA Gliadin<sup>DP</sup> IgA is intended for the in vitro quantitative measurement of IgA antibodies directed to gliadin in human serum or plasma to aid in the diagnosis of celiac disease. EliA Gliadin<sup>DP</sup> IgA is to be used together with the EliA IgA method on the instrument Phadia 100.

**SUMMARY AND EXPLANATION OF THE TEST**

Celiac disease is a life-long condition in which ingestion of gluten, the water insoluble wheat-gliadin and the prolamins in rye and barley, leads to chronic inflammation and damage of the small intestinal mucosa. The disease is multifaceted in nature with clinical presentation ranging from gastrointestinal manifestations to asymptomatic, silent and extraintestinal forms.<sup>1</sup> It is widely accepted that dermatitis herpetiformis, a bullous skin disease, is induced by gluten.<sup>2</sup> The term gluten refers to a whole set of proteins in the so-called endosperm, the nutritive tissue of the grain seed of wheat, rye, oats and barley. The alcohol-soluble polypeptides of gluten, the gliadins, are solely responsible for the toxic effects to the intestinal mucosa.<sup>3</sup> More recent research revealed that gliadin peptides deamidated by tissue transglutaminase represent more specific B-cell epitopes than native peptides.<sup>4,5</sup> Further studies showed that increased specificity can also be observed for anti-gliadin assays based on deamidated peptides.<sup>6,7</sup>

**PRINCIPLES OF THE PROCEDURE**

The EliA Gliadin<sup>DP</sup> IgA Wells are coated with synthetic deamidated gliadin peptides. If present in the patient's specimen, antibodies to the gliadin peptides bind to their specific antigen. After washing away non-bound antibodies, enzyme-labeled antibodies against human IgA antibodies (EliA IgA Conjugate) are added to form an antibody-conjugate complex. After incubation, non-bound conjugate is washed away and the bound complex is incubated with a Development Solution. After stopping the reaction, the fluorescence in the reaction mixture is measured. The higher the response value, the more specific IgA is present in the specimen. To evaluate test results, the response for patient samples is compared directly to the response for calibrators.

**REAGENTS / MATERIAL**

The EliA reagents are available as modular packages, each purchased separately. All packages except for the EliA Celiac Positive Control 100 and the EliA IgG/IgM/IgA Negative Control 100 are required to carry out an EliA Gliadin<sup>DP</sup> IgA Test. The EliA Gliadin<sup>DP</sup> IgA Wells are packed in carriers which are stored in sealed aluminium foil bags containing a desiccant.

**EliA Gliadin<sup>DP</sup> IgA Test-Specific Reagents****EliA Gliadin<sup>DP</sup> IgA Well (Art. No. 14-5538-01)**

Gliadin <sup>DP</sup> IgA Well; short name: Agp	coated with synthetic deamidated gliadin peptides	4 carriers (12 wells each); sufficient for 48 determinations	ready for use; store dry at 2-8 °C until expiration date
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**EliA Celiac Positive Control 100 (Art. No 83-1041-01)**

Human serum in PBS containing BSA, detergent and sodium azide (0.095 %); symbol: pos	Multiparameter control containing IgG and IgA antibodies to tTG and gliadin	6 single-use vials (0.3 ml each); sufficient for 2 determinations per vial	Ready for use; store at 2-8 °C until expiration date
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EliA Celiac Positive Control 100 is prepared from selected pooled human sera.

**EliA IgG/IgM/IgA Negative Control 100 (Art. No 83-1042-01)**

Human serum in PBS containing BSA, detergent and sodium azide (0.095 %); symbol: neg	Multiparameter control containing normal sera from healthy donors	6 single-use vials (0.3 ml each); sufficient for 2 determinations per vial	ready for use; store at 2-8 °C until expiration date
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EliA IgG/IgM/IgA Negative Control 100 is prepared from selected pooled human sera.

**EliA Method-Specific Reagents (Phadia 100)****EliA Sample Diluent (Art. No 83-1003-01)**

Sample Diluent (yellow colored); PBS containing BSA, detergent and sodium azide (0.095 %)	6 vials (9 ml each); sufficient for 6 x 30 dilutions (instrument 1:100 dilution)	ready for use; store at 2-8 °C until expiration date
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**EliA IgA Conjugate (Art. No 83-1014-01)**

IgA Conjugate (blue colored); β-Galactosidase anti-IgA (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06 %); symbol: EI-A	6 vials (4.8 ml each); sufficient for 6 x 48 determinations	ready for use; store at 2-8 °C until expiration date DO NOT FREEZE
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**EliA IgA Conjugate (Art. No 83-1012-01)**

IgA Conjugate (blue colored); β-Galactosidase anti-IgA (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06 %); symbol: EI-A	2 vials (4.8 ml each); sufficient for 2 x 48 determinations	ready for use; store at 2-8 °C until expiration date DO NOT FREEZE
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**EliA IgA Calibrators (Art. No 83-1013-01)**

human IgA (0, 0.3, 1.5, 5, 15, 80 µg/l); in PBS containing BSA, detergent and sodium azide (0.095 %) symbol: CAL-0, CAL-0.3, CAL-1.5, CAL-5, CAL-15, CAL-80	6 single-use vials (0.3 ml each); sufficient for one calibration curve	ready for use; store at 2-8 °C until expiration date
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Manufactured from human sera.

### EliA IgA Curve Control (Art. No 83-1010-01)

human IgA (5 µg/l); in PBS containing BSA, detergent and sodium azide (0.095 %) symbol: CC-1	6 single-use vials (0.3 ml each); sufficient for 6 runs	ready for use; store at 2-8 °C until expiration date
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Manufactured from human sera.

### EliA IgA Calibrator Well (Art. No 14-5516-01)

IgA Calibrator Well coated with mouse monoclonal antibodies; short name: Acal	4 carriers (12 wells each); sufficient for 48 determinations	ready for use; store dry at 2-8 °C until expiration date
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### EliA Dummy Well (Art. No 14-5510-01)

Dummy Well required by the Phadia 100 System for empty run positions	4 carriers (12 wells each); sufficient for 48 positions	ready for use; store dry at 2-8 °C until expiration date
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### Phadia 100 General Reagents

#### Development Solution (Art. No. 10-9478-01)

Development Solution 0.01 % 4-Methylumbelliferyl-β-D-galactoside, <0.0010 % preservative**	6 vials (6 ml each); reagents for 6 x 48 determinations	ready for use; store at 2-8 °C until expiration date. DO NOT FREEZE
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#### Stop Solution (Art. No. 10-9479-01)

Stop Solution 4 % Sodium Carbonate	6 bottles (65 ml each); *reagents for 6 x 240 determinations	ready for use; store at 2-8 °C until expiration date
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\* Due to different ImmunoCAP and EliA assay processes, a high residual volume is to be expected.

\*\* Preservative: mixture of 5-chloro-2-methyl-2H-isothiazol-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1).

#### Washing Solution (Art. No. 10-9422-01/10-9202-01)

For information see separate Washing Solution package insert.

The expiration date for each of the complete packages is stated on the outer label. However, each component is stable until the date stated on the respective vial label.

**Material not provided:** purified water, graduated cylinder

### WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use reagents beyond their expiration dates.
- We do not recommend to pool reagents.
- Wear gloves while handling samples and reagents provided.
- Some of the reagents are manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, for antibodies to HIV1, HIV2 and hepatitis C virus and found negative. Nevertheless, all recommended precautions for the handling of blood derivatives should be observed. Please refer to Human Health Service (HHS) Publication No. (CDC) 93-8395 or local and national guidelines on laboratory safety procedures.

**WARNING!** Reagents contain sodium azide (NaN<sub>3</sub>) as a preservative. NaN<sub>3</sub> may be toxic if ingested or absorbed by skin or eyes. NaN<sub>3</sub> may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local and national guidelines.

Waste Bottle and ImmunoCAP/EliA Well Waste Container may be contaminated by potentially infectious material. Use appropriate safety measures and wear gloves.

### Indication of Instability

Phadia 100 Instrument Software has built-in acceptance limits for the calibration curve and the curve control. EliA Wells are moisture sensitive. An activity loss that might occur due to inappropriate handling can be detected using the appropriate EliA Control. For more information see Phadia 100 User's Guide/Reference Manual.

### INSTRUMENT

EliA reagents are to be used with the Phadia 100 Instrument Software version 2.1 or higher. The Phadia 100 Instrument processes all steps of the test and prints results automatically after the test is completed. For further information regarding test set-up, instrumentation and software etc. see Phadia 100 User's Guide/Reference Manual.

### SPECIMEN COLLECTION, HANDLING AND PREPARATION

The procedure can be performed with serum or plasma specimens. Lipemic, hemolyzed or microbially contaminated samples may give poor results and should not be used. Avoid repeated freezing and thawing. Samples should be stored in aliquots at -20 °C (-4 °F) or below for repeated measurements.

### Sample Dilution

Samples must be diluted with EliA Sample Diluent. A 1:50 dilution of the samples is required for the EliA Gliadin<sup>DP</sup> IgA Test. Samples can be diluted manually, but instrument dilution is recommended and is a default setting in Phadia 100 Instrument Software.

### PROCEDURE

#### Handling of EliA Gliadin<sup>DP</sup> IgA Well

Prior to opening the foil bag, equilibrate to room temperature. For stability reasons the carriers have to be put back in the desiccant-containing foil bag directly after dispensing the wells. Because it is important to store the wells in dry conditions at 2-8 °C, the bag must be properly resealed. Shelf-life after first opening: 9 months, if not limited by expiry date stated on the carrier and foil bag.

#### Lot-specific code of EliA Gliadin<sup>DP</sup> IgA Well

Make sure to enter the lot-specific code of the EliA Gliadin<sup>DP</sup> IgA Well. This code is stated on the carrier and foil bag as **Code**, and it is encoded within the barcode of the foil bag. Preferably use a barcode reader.

#### Lot-specific code of EliA IgA Calibrator Well

Make sure to enter the lot-specific code of the EliA IgA Calibrator Well. This code is stated on the carrier and foil bag as **Code**, and it is encoded within the barcode of the foil bag. Preferably use a barcode reader.

### Lot specific code of EliA IgA Conjugate

Make sure to enter the lot-specific Calibration Code of the IgA Conjugate given on the box and the vial as **CalCode**, and encoded within the barcode of the IgA Conjugate. Preferably use a barcode reader to enter the Calibration Code.

### Volumes per determination

#### Reagent volumes per determination

Calibrator	90 µl
EliA IgA Conjugate	90 µl
Development Solution	90 µl
Stop Solution	200 µl

#### Sample volumes per determination

Manual dilution	90 µl of diluted sample
Instrument dilution (1:50)	22 µl of non diluted sample

For tube-specific dead volumes see Phadia 100 User's Guide/Reference Manual.

#### Reagent volumes per run

Washing Solution	1 l*
Rinse Solution	1 l*

\* The residual volume depends on the number of samples and dilution method used.

### Procedural comments

- From one sample diluted by the instrument (1:50), up to 5 determinations can be made.
- When using software default, samples are run in single determination.
- Washing Solution must be at room temperature when used.
- Total time is 2.5 hours for one test run processing 48 wells.
- Incubations are automatically performed at 37 °C (98.6 °F).
- Software ≥ 2.1 / 022: If you want to perform more than one test per patient you can also use the following predefined test panels:

Panel	Description	EliA tests included
pcd	Celiac disease	Celikey IgA, Celikey IgG, Gliadin <sup>DP</sup> IgA, Gliadin <sup>DP</sup> IgG
pca	Celiac disease IgA	Celikey IgA, Gliadin <sup>DP</sup> IgA
pgl	Gliadin	Gliadin <sup>DP</sup> IgA, Gliadin <sup>DP</sup> IgG
pcag	tTG IgA, Gliadin IgG	Celikey IgA, Gliadin <sup>DP</sup> IgG

### CALIBRATION AND REFERENCE MATERIAL

The calibration curve is obtained with EliA IgA Calibrators which are run in duplicate. The curve is stored and subsequent tests are evaluated against the stored curve using only the EliA IgA Curve Control (run in duplicate). The IgA Calibrators are traceable via an unbroken chain of calibrations to the International Reference Preparation (IRP) 67/86 of Human Serum Immunoglobulins A, G and M from World Health Organization (WHO).

**There are no international standards for Gliadin antibodies. Results are given in arbitrary EliA Units/ml.**

### A new calibration curve must be run when:

- the last calibration was made more than one month ago or
- a new lot of EliA IgA Conjugate is introduced or
- when the EliA IgA Curve Control is outside the specified limits (defined in Phadia 100 Instrument Software).

### QUALITY CONTROL

#### Record Keeping

It is good laboratory practice to record the lot numbers of the components used, the dates when they were first opened and remaining volumes.

#### Control Specimens

Good laboratory practice requires that quality control specimens should be included in every run. Any material used should be assayed repeatedly to establish mean values and acceptance ranges. EliA Controls are available for the quality control of the measurements.

### CALCULATION AND INTERPRETATION OF RESULTS

#### Presentation of Results

Phadia 100 measures specific IgA concentrations in µg/l. By using a conversion factor given by the lot-specific code of the EliA Gliadin<sup>DP</sup> IgA Well, the results are automatically converted to EliA U/ml.

#### Interpretation of Test Results

The ranges (negative, equivocal, positive) recommended for the evaluation of the results are given in the table below.

Test	Unit	negative	equivocal	positive
EliA Gliadin <sup>DP</sup> IgA	EliA U/ml	<7	7–10	>10

Good laboratory practice requires that each laboratory establishes its own range of expected values.

### LIMITATIONS

A definitive clinical diagnosis should not be based on the results of a single diagnostic method, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

### EXPECTED VALUES

Antibody prevalence in autoimmune patients varies widely depending on disease area. The proportion of sera from a normal population found positive for gliadin antibodies covered by the EliA Gliadin<sup>DP</sup> IgA test is below 1 %. Expected values may vary depending on the population tested.

## Results Obtained for Healthy Subjects

The frequency distribution for Gliadin IgA antibodies was investigated in a group of apparently healthy subjects equally distributed by age and gender, using sera from a Caucasian population obtained from a blood bank. The results are given in the table below.<sup>(1)</sup>

Test	Unit	No. of samples	Mean Value	95%-percentile	99%-percentile
EliA Gliadin <sup>DP</sup> IgA	EliA U/ml	400	1.7	3.5	10.4

## PERFORMANCE CHARACTERISTICS

### Measuring Range

The measuring range (detection limit, upper limit) for EliA Gliadin<sup>DP</sup> IgA is from 0.1 to  $\geq 142$  EliA U/ml. No hook effects could be observed for concentrations up to 10 fold above the measuring ranges.<sup>(1)</sup>

Only values above the Detection Limit can be regarded as valid results. The upper limit of the reported results can vary due to a lot-specific conversion from  $\mu\text{g/l}$  to EliA U/ml. Results above the upper limit are reported as "above".

Please note that due to differing binding characteristics of the antibodies in patient samples, not all sera can be diluted linearly within the measuring range.

### Specificity

The EliA Gliadin<sup>DP</sup> IgA Test permits the determination of IgA antibodies directed against the gliadin antigen as described in section "Reagents".

### Precision

To determine the precision of the assay, the variability was assessed in a study with 18 runs by examining the samples in 108 replicates on 3 instruments over 6 days with a calibration curve in each run. The statistical evaluation was performed by Analysis of Variance. The results are given in the table below.<sup>(1)</sup>

Test	Sample	Unit	Mean Value	Coefficients of variation (%)	
				Intra-Run	Inter-Run
EliA Gliadin <sup>DP</sup> IgA	1	EliA U/ml	7.4	3.8	1.8
	2	EliA U/ml	11.3	4.2	2.4
	3	EliA U/ml	22.7	4.4	1.2

## WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Phadia AB may affect the results, in which event Phadia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Phadia AB and its authorized distributors, in such event, shall not be liable for damages, indirect or consequential.

<sup>(1)</sup> Studies performed at Phadia GmbH, Freiburg, Germany

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 <b>LOT</b>	Batch code		Contains sodium azide
	Biological Risk		Contains x determinations
	Store at 2-8°C/35-46°F		Read Directions for Use
	Expiration date		Manufactured by
 <b>IVD</b>	For <i>in vitro</i> diagnostic use		Do not reuse in a second run

 **Phadia AB**  
SE-75 137 Uppsala, Sweden  
Phone: +46-18-16 50 60 · Fax: +46-18-14 03 58  
E-mail: [autoimmunity@phadia.com](mailto:autoimmunity@phadia.com) / Internet: [www.phadia.com](http://www.phadia.com)

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