

# Celiac EmA (Anti-Endomysium Antibody) IgG ELISA

Catalog Number:  
CEG31-K01

Enzyme immunoassay for the determination of **IgG antibodies** to human **endomysial autoantigens** in human serum



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v. 1.0*

## INTENDED USE

**Celiac EmA (Anti-Endomysium Antibody) IgG ELISA assay kit is used for the quantitative determination of IgG autoantibodies to human endomysial autoantigens (EmA).**

Several autoantigens have been discussed for celiac disease and Dermatitis herpetiformis recently. Dieterich et al. have described two non-collagenous proteins with an apparent molecular weight of 90 and 300 kDa being the main autoantigens detected by EmA (1). Celiac disease, or gluten-sensitivity, is found already in neonates and is characterized by small intestinal damages leading to a so-called "flat" mucosa. Due to this extensive lesions mal-absorption occurs frequently accompanied with a depletion of key nutrients.

Incidence rates for celiac disease range from 1 in 300 (Western Ireland) to 1 in 4700 in European countries. However, a high number of subclinical cases of celiac disease have been detected by in-vitro tests revealing a prevalence of 4 in 1000. Individuals suffering from prolonged celiac disease additionally face an elevated risk of developing T cell lymphoma. Dermatitis herpetiformis is an autoimmune chronic relapsing skin disease characterized by formation of sub-epidermal blisters. Deposits of IgG antibodies are found in the basal membrane associated with neutrophil and eosinophil infiltration. Dermatitis herpetiformis seems to demonstrate celiac like changes in the intestine resembling the inflammatory process in celiac samples.

## PRINCIPLE OF THE TEST

Celiac EmA (Anti-Endomysium Antibody) IgG ELISA assay kit is an enzyme immunoassay for the quantitative determination of IgG autoantibodies to the supposed major autoantigens in Dermatitis herpetiformis and celiac disease in sample serum.

Autoantibodies of the diluted samples and standards react with the immobilized human autoantigens on the solid phase of the microtiter plate. Celiac EmA (Anti-Endomysium Antibody) IgG ELISA assay kit guarantees the specific binding of autoantibodies usually found by immunofluorescence on endomysial antigens. Following an incubation period of 60 min at room temperature (18...25°C), unbound serum components are removed by a wash step.

The bound autoantibodies react specifically with anti-human-IgG-antibodies conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at room temperature. Excessive conjugate is separated from the solid-

phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. This enzyme reaction is stopped by dispensing an acidic solution (H<sub>2</sub>SO<sub>4</sub>) into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the concentrations of the antibodies of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve. Evaluating the test by a semi-quantitative method is also possible.

## SAMPLES

### Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, hemolytic and contaminated samples should not be used.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

### Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

The samples may be kept at 2 - 8 °C for up to two days. Long-term storage requires - 20 °C.

## TEST COMPONENTS FOR 96 DETERMINATIONS

<b>A</b>	<b>Microtiter plate</b> , 12 breakable strips per 8 wells (total 96 individual wells) coated with purified human EmA autoantigen and gliadin fragments	1 vacuum sealed with desiccant
<b>Ag</b> <b>96</b>		
<b>B</b>	<b>Concentrated wash buffer</b> sufficient for 1000 ml solution	100 ml concentrate capped white
<b>BUF</b> <b>WASH</b> <b>10x</b>		
<b>C</b>	<b>Sample diluent</b>	100 ml ready for use capped black
<b>DIL</b>		

<b>D</b>	<b>Conjugate</b> containing anti-human-IgG- (sheep) coupled with HRP	15 ml ready for use capped red
<b>CONJ</b>		
<b>E</b>	<b>Substrate</b> 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
<b>SOLN</b> <b>TMB</b>		
<b>F</b>	<b>Stop solution</b> 0.25 M sulfuric acid	15 ml ready for use capped yellow
<b>H2SO4</b> <b>0.25M</b>		
<b>0 - 4</b>	<b>Calibrators</b> (diluted serum) conc.: 1, 10, 30, 100, 300 U/ml	1 ml each ready for use capped white
<b>CAL</b>		
<b>P</b>	<b>Positive control</b> (diluted serum) conc.: see leaflet enclosed	1 ml ready for use capped red
<b>CONTROL</b> <b>+</b>		

### Materials required in addition

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
- pipette tips
- trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- graduated cylinders
- tubes (2 ml) for sample preparation
- distilled or de-ionized water

### Size and storage

Celiac EmA (Anti-Endomysium Antibody) IgG ELISA assay kit has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of Celiac EmA (Anti-Endomysium Antibody) IgG ELISA assay kit have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

## Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water. For example, dilute 8 ml of the concentrate with 72 ml of distilled water per strip. The wash solution prepared is stable up to 30 days at 2 - 8 °C.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

## ASSAY PROCEDURE

- **Dilute sample sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl serum + 1.0 ml sample diluent (C).**
- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently, avoid foam.
2. Dispense  
**100 µl** calibrators 0 - 4 (quantitative) or **100 µl** of calibrator 1 (semi-quantitative)  
**100 µl** positive control  
**100 µl** diluted samples  
into the respective wells.
3. Seal plate, incubate **60 min** at room temperature.
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Seal plate, incubate **30 min** at room temperature.
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **15 min** protected from light at room temperature.
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

## DATA PROCESSING

Celiac EmA (Anti-Endomysium Antibody) IgG ELISA assay kit allows both the quantitative (5 calibrators) and semi-quantitative (calibrator 1 as cut-off calibrator) evaluation of the results.

### Quantitative evaluation

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 4 (CAL 0 optionally) on the ordinate, y-axis, (lin. scale) versus their respective concentrations on the

abscissa, x-axis, (log. scale). Anti- IgG concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

Using the recommended dilution of 1 + 100 (v/v) for samples sera, no correction factor is necessary, as all other components of the Celiac EmA (Anti-Endomysium Antibody) IgG ELISA assay kit are supplied accordingly.

### Semi-quantitative evaluation

Results can be calculated semi-quantitatively using the binding index BI (ratio) between the optical density of an unknown sample and the optical density of **calibrator 1** (10 U/ml):

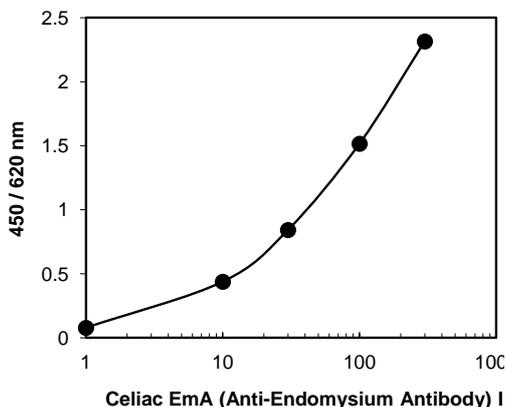
$$BI = OD_{\text{Sample}} / (OD_{\text{Calibrator 1}} \times 1,5)$$

Both evaluation variants of Celiac EmA (Anti-Endomysium Antibody) IgG ELISA assay kit may be achieved also with computer assisted analysis software intergrated in the photometers.

#### Example of typical assay results

well	OD (a)	OD (b)	OD (mean)	U/ml
Cal 0	0.072	0.084	0.078	1
Cal 1	0.425	0.450	0.438	10
Cal 2	0.825	0.859	0.842	30
Cal 3	1.501	1.530	1.516	100
Cal 4	2.308	2.322	2.315	300
patient 1	1.241	1.260	1.250	65

#### TYPICAL STANDARD CURVE



### Test validity

The test run is valid if:

- the mean OD of the standard 1 is  $\leq 0.7$
- the mean OD of the standard 4 is  $\geq 1.2$

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

### REFERENCE VALUES

Celiac EmA (Anti-Endomysium Antibody) IgG ELISA	U/ml	BI
negative	< 20	< 1
positive	$\geq 20$	$\geq 1$

### Limitations of Method

Healthy individuals should be tested negative by CeliAK EmA human IgG. However, anti-endomysial IgG autoantibody positive apparently healthy persons do occur.

### CHARACTERISTIC ASSAY DATA

#### Calibration

Due to the lack of an international reference material the Celiac EmA (Anti-Endomysium Antibody) IgG ELISA assay kit is calibrated in arbitrary units (U/ml).

#### Linearity

Dilutions of selected positive specimens in anti-endomysial IgG autoantibody free human serum are determined according to their expected theoretical values with Celiac EmA (Anti-Endomysium Antibody) IgG ELISA assay kit.

#### Sensitivity

The analytical sensitivity of the Celiac EmA (Anti-Endomysium Antibody) IgG ELISA assay kit is 3 U/ml.

#### Precision

Intraassay (n = 8)		Interassay (n = 4 x 8)	
mean (U/ml)	CV %	mean (U/ml)	CV %
198.1	5.96	214.9	6.10
61.5	4.73	63.8	7.35
29.5	2.36	32.6	6.33
15.8	3.49	14.8	7.89

## INCUBATION SCHEME

# Celiac EmA (Anti-Endomysium Antibody) IgG ELISA

<b>Dilute sample</b>	<b>10 µl serum + 1.0 ml sample diluent (C)</b>
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1	Bring all ready for use reagents to room temperature (18...25°C) before use.				
			calibrators	control	sera
2	Pipette	Calibrators (0 - 4) or Calibrator 1 Positive Control (P) prediluted 1 + 100 sample sera	100 µl	100 µl	100 µl
3	Incubate	60 minutes at room temperature			
4	Wash	Decant, Dispense 3 x 300 µl (made of B)			
5	Pipette conjugate (D)		100 µl	100 µl	100 µl
6	Incubate	30 minutes at room temperature			
7	Wash	Decant, Dispense 3 x 300 µl (made of B)			
8	Pipette substrate (E)		100 µl	100 µl	100 µl
9	Incubate protected from light	15 minutes at room temperature			
10	Pipette stop solution (F)		100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm				

## SAFETY PRECAUTIONS

- **This Celiac EmA (Anti-Endomysium Antibody) IgG ELISA assay kit is for research use only.** Follow the working instructions carefully. Eagle Biosciences 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.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.

- Since the Celiac EmA (Anti-Endomysium Antibody) IgG ELISA assay kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

### **3Warranty Information**

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*For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at [info@eaglebio.com](mailto:info@eaglebio.com) or at 866-411-8023.*