



EAGLE
BIOSCIENCES

Anti-Gliadin sIgA / IgA ELISA

Catalog Number: GLI35-K01

96 Wells

For Research Use Only.

v. 2.0 (08.11.22)

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INTENDED USE

The Eagle Biosciences Anti-Gliadin sIgA / IgA ELISA is intended for the quantitative determination of Anti-Gliadin sIgA / IgA in stool. The Anti-Gliadin sIgA / IgA ELISA kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

Celiac disease is a chronic illness of the small intestinal mucous membrane. It is caused by an intolerance against gluten, which is found in many cereals. The intake of gluten-containing food leads to inflammation of the small intestinal mucous membrane. The resorption of nutrients is therefore reduced. The symptoms of the disease are reduction of weight, diarrhoea, vomiting, anorexia and tiredness. The growth in children is reduced. The only therapeutic treatment is a gluten-free diet.

An untreated celiac disease has been known to increase the risk of non-Hodgkin-lymphoma and colon cancer. In five to ten percent of the patients, celiac disease is associated with diabetes mellitus type 1. Women are more often affected than men. The outcome of the disease is pronounced during infancy and in an age between 30 and 40 years.

The Eagle Biosciences Anti-Gliadin sIgA / IgA ELISA kit allows for an easy, rapid and precise quantitative determination of the secretory IgA and IgA gliadin antibodies in stool. The kit includes all reagents ready to use for preparation of the samples.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are strictly intended for Research Use Only.
- Do not interchange kit components from different lots.
- The stop solution (STOP) contains acid and has to be handled carefully. It is corrosive and causes burns. It should be handled with gloves, eye protection, and appropriate protective clothing in a hood. Any spill should be wiped out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation. In case of an accident or indisposition contact immediately a physician.
- The substrate TMB (tetramethyl benzidine) is toxic by ingestion and contact with the skin. Any spill should be wiped out immediately with copious quantities of water.
- Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
- The reagents of the Anti-Gliadin sIgA / IgA ELISA kit contain bactericides to protect against bacterial growth. Avoid the contact with the skin or mucous membrane.
- Reagents should not be used beyond the expiration date shown on kit label.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.



MATERIALS PROVIDED

Catalog #	Component	Description	Amount
IC6300mtp	MTP	Microtiter plate coated	12 x 8 wells
IC6300wp	WASHBUF	Anti-gliadin / anti-transglutaminase ELISA wash buffer conc. 10 fold	100 ml
IC6300pb	SAMPLEBUF	Sample buffer	500 ml
IC6300st	STD	Standard (0.5 ml lyoph.) (25; 50; 100; 200 mU/g)	4 vials
IC6300ko	CTRL	Controls (2 levels, 0.5 ml lyoph.)	1 vial each
IC6300kg	CONJ	Conjugate, peroxidase-labelled antibody	15 ml
IC6000su	SUB	TMB substrate (tetramethyl benzidine)	15 ml
IC6300sp	STOP	Stop solution	10 ml

MATERIALS REQUIRED BUT NOT PROVIDED

- Laboratory balance
- Centrifuge, 3000xg
- Glass or plastic vials
- Various pipettes
- Foil to cover the microtiter plate
- Multichannel or multi-pipette
- ELISA reader with filter 450 nm (reference filter 620 or 690 nm)
- Microtiter plate shaker
- Vortex mixer

REAGENT PREPARATION

Microtiter Plate (MTP): Take the needed strips out of the bag and mount them on the holder. Please take care that the package has reached room temperature before opening the bag. Strips which are not needed could be stored at 2-8°C. Please dispose the holder when all strips are used.

Standards (STD) and Controls (CTRL): Re-suspend the standards and controls in 0.5 ml deionized or distilled water. Please take the 1:10 diluted wash buffer concentrate as 0-standard. The re-suspended standards and controls can be frozen only once.

Wash buffer (WASHBUF): Dilute the wash buffer concentrate 1:10 with deionized or distilled water (1 part buffer + 9 parts water). The dilution is stable for 14 days at 2-8°C.

Important: When storing the wash buffer concentrate at 2-8°C crystallization could occur. Before dilution all crystals must be dissolved.



It is recommended to dilute only the amount of buffer which is used to process the given samples. All other test reagents are stable at 2-8 °C. until the expiry date stated on the label.

SPECIMEN COLLECTION

Stool samples:

The antibodies are extracted by the sample buffer out of the stool sample.

- 100 mg stool are mixed with 5 ml SAMPLEBUF on a vortex mixer until the mixture is homogenous.
- 1 ml of the mixture is transferred into an "Eppendorf" reaction vial and centrifuged for 30 min at 3000xg or 5 min at 10000xg.

PROCEDURE

1. The Anti-Gliadin sIgA / IgA ELISA kit determines human anti-gliadin sIgA / IgA antibodies according to the "sandwich"-principle.
2. Anti-gliadin antibodies in sample, standard and controls bind to gliadin, which is coated to the microtiter plate.
3. After a washing step a peroxidase labeled detection antibody is added. A second washing step is followed by the addition of the substrate which is converted to a colored product by the peroxidase.
4. The reaction is terminated by the addition of an acidic stop solution.
5. The optical densities are read at 450 nm in a microtiter plate reader.
6. The anti-gliadin concentration can be calculated from the standard curve.

SAMPLE PREPARATION

All reagents and samples should have room temperature (18-26°C) and should be mixed well before use. The position of standards, controls, and samples are noted on a protocol sheet.

1. Take out the needed strips of the microtiter plate and wash 1x with 250 µl diluted WASHBUF. Remove residual buffer by tapping the plate on absorbent paper after the washing step.
2. Pipette **100µl STD, CTRL** and **samples** in duplicate in the microtiter plate. For standard 0 use the diluted WASHBUF. The strips are covered and incubated for **60 min** at room temperature (18-26 °C).
3. Discard the content of the microwells and wash 5x with 250 µl diluted WASHBUF. Remove residual buffer by tapping the plate on absorbent paper after the last washing step.
4. Pipette **100 µl CONJ** in each microwell. The strips are covered and incubated for **60 min** at room temperature (18-26 °C).



5. Discard the content of the microwells and wash 5x with 250 μ l diluted WASHBUF. Remove residual buffer by tapping the plate on absorbent paper after the last washing step.
6. Pipette **100 μ l SUB** in each microwell. Incubate for **10-15 min** at room temperature (18-26 °C) in the dark.
7. Pipette **50 μ l STOP** in each microwell. Mix well.
8. Read the absorbance at 450 nm. If the microtiter plate reader allows to use a reference wavelength use 620 or 690 nm as reference wavelength. Reading should be done within 5 min after stopping reaction. If the highest standard exceeds the range of the reader the measurement should be done at 405 nm against 620 nm (690 nm).

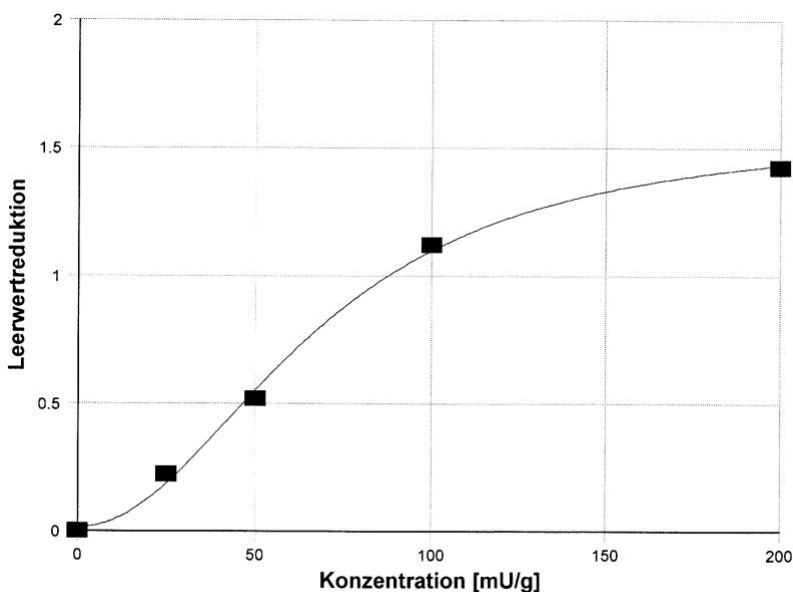
CALCULATIONS

For calculating the results, we recommend using the 4-parameter algorithm. If this algorithm is not available a "point to point" or a "spline" function can be used.

Stool samples

The anti-gliadin concentration is read from the standard curve.

Typical Standard Curve



■ Grp. 1: A=0.014934 B=2.0898 C=68.101 D=1.5834 d=0.024797 r=0.99894

The curve given above is only for demonstration. It must not be used for calculation of your samples



INTERNAL QUALITY CONTROL

Reference values:

Stool: < 100 mU/g

We recommend that each laboratory should develop their own normal range. The values mentioned above are only for orientation and can deviate from other published data.

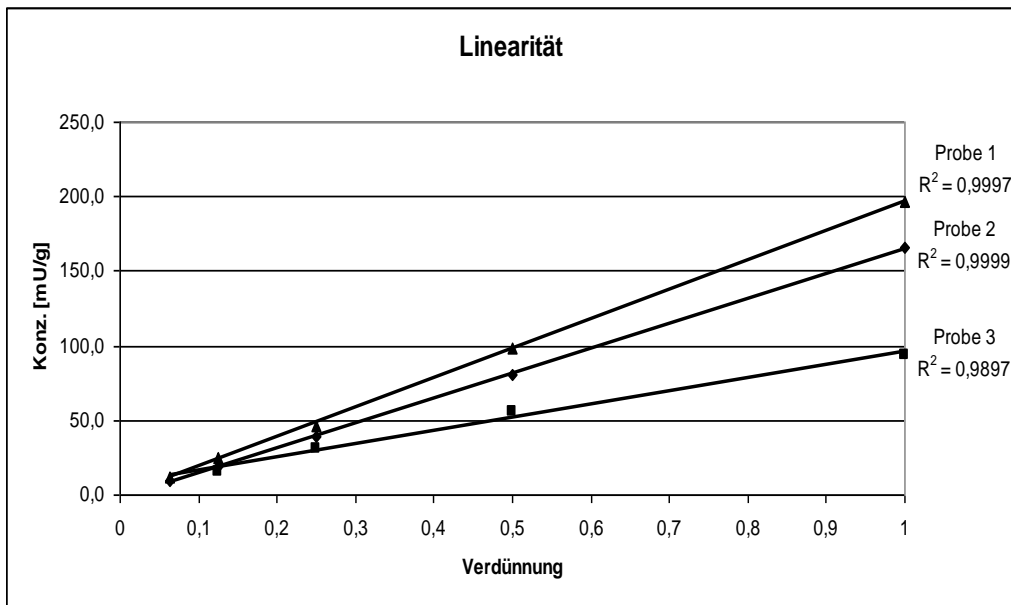
VALIDATION DATA

Precision and reproducibility

Intra-Assay CV:	3.7 % (21.5 mU/g)	[n = 10]
	4.6 % (47.0 mU/g)	[n = 10]
Inter-Assay CV:	3.1 % (79.7 mU/g)	[n = 10]
	9.2 % (22.5 mU/g)	[n = 10]
	9.3 % (48.0 mU/g)	[n = 10]
	8.8 % (83.5 mU/g)	[n = 10]

LINEARITY

Sample	Dilution factor	Expected [mU/g]	Measured [mU/g]	Recovery [%]
1	--	--	165.6	
	1:2	82.8	80.4	97.1
	1:4	41.4	39.1	94.4
	1:8	20.7	19.3	93.2
	1:16	10.4	8.9	86.0
2	--	--	93.4	
	1:2	46.7	56.0	119.9
	1:4	23.4	31.0	132.8
	1:8	11.7	15.5	132.8
3	--	--	196.3	
	1:2	98.2	98.5	100.4
	1:4	49.1	46.3	94.3
	1:8	24.5	25.0	101.9
	1:16	12.3	11.3	92.1



DETECTION LIMIT

Stool: 1.3 mU/g

For the determination the zero-standard was measured 20 times. The 2-fold standard deviation was added to the mean value of the optical density. The respective concentration was read from the standard curve.

RECOVERY

Sample	Endogen [mU/g]	Added	Expected [mU/g]	Measured [mU/g]	Recovery [%]
1	11.4	20.0	31.4	29.4	93.6
		50.0	61.4	66.7	108.6
		75.0	86.4	87.4	101.2
2	10.8	20.0	30.8	28.3	91.9
		50.0	68.8	66.2	108.9
		75.0	85.8	84.1	98.0

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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 or at 866-411-8023.