Anti-Gliadin sIgA / IgA ELISA

For the determination of anti-gliadin sIgA / IgA in human stool

Ship and store standards and controls at ≤ -16˚C.

For Research Use Only. Not for Use in Diagnostic Procedures.

Catalog Number: 31-GLIHU-E01

Size: 96 determinations

Version: 05.12.2025 / ALPCO 1.1

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INTENDED USE

The Anti-Gliadin sIgA / IgA ELISA is intended for the quantitative determination of anti-gliadin sIgA / IgA in human stool. For Research Use Only. Not for Use in Diagnostic Procedures.

INTRODUCTION

Gluten proteins are major storage proteins in the endosperm of wheat, barley, and rye grains. They are highly resistant to hydrolysis in the gastrointestinal tract. Wheat gluten is comprised of gliadin monomers and glutenin polymers.[1,2] This kit detects the presence of antibodies to the gliadin monomers.

PRINCIPLE OF THE ASSAY

The anti-gliadin-ELISA test determines human anti-gliadin sIgA / IgA antibodies according to the sandwich principle. Anti-gliadin antibodies in sample, standard, and controls bind to gliadin, which is coated to the microtiter plate. After a wash step, a peroxidase-labeled detection antibody is added. A second wash step is followed by addition of the substrate which is converted to a colored product by peroxidase. The reaction is terminated by the addition of an acidic stop solution. The optical densities are measured at 450 nm (against the reference wavelength 620 nm) in a microtiter plate reader. The anti-gliadin concentration can be calculated from the standard curve.

Calibration: Calibration was done in relative units as no higher order reference material is available.

MATERIALS SUPPLIED

31-GLIHU-E01			
Component	**Quantity**	**Preparation**	**Storage**
Microtiter plate, coated	1 plate: 12 x 8-well strips	Ready-to-use	2 - 8˚C
ELISA Wash Buffer concentrate, 10X	100 mL	Dilute 1:10	2 - 8˚C
Standards*	4 vials, 0.5 mL each	Ready-to-use	**≤ -16˚C**
Control 1 and 2*	2 vials, 0.5 mL each	Ready-to-use	**≤ -16˚C**
Conjugate: peroxidase-labeled antibody	15 mL	Ready-to-use	2 - 8˚C
Sample buffer	500 mL	Ready-to-use	2 - 8˚C
TMB Substrate: tetramethylbenzidine	15 mL	Ready-to-use	2 - 8˚C
Stop solution	10 mL	Ready-to-use	2 - 8˚C
Plate Sealers	2		

*Please refer to the Certificate of Analysis enclosed with each kit for Standard and Control concentrations.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Centrifuge, 3000 x g

- Plastic vials
- Stool sample extraction vials (80-EXDEV2-100)
- Various pipettes
- Multichannel or multipipette
- Foil to cover the microtiter plate (substrate step)
- Distilled or deionized water
- ELISA reader with 450 nm filter (620 nm reference filter)
- Microtiter plate shaker (2 mm orbital shaker capable of 400 rpm)
- Vortex mixer
- Absorbent paper (lint-free)
- Timer

PRECAUTIONS

- This assay is for Research Use Only. Not for use in diagnostic procedures.
- Individual components from different batches and kits should not be interchanged. The expiry dates on the packaging must be observed.
- The kit reagents contain preservatives to protect against bacterial growth. Contact with the skin and/or mucous membranes should be avoided.
- The kit contains components of human origin. The starting reagents were tested for antibodies against HIV1/2, hepatitis B, and anti-HCV using immunoassay methods. All parameters tested were found negative. As a precautionary measure, all kit reagents should always be treated as potentially infectious material in accordance with health care accident prevention regulations.
- The substrate TMB (tetramethylbenzidine) is toxic by ingestion and skin contact. In the event of skin contact, the affected area must be washed immediately with plenty of water and soap.
- Avoid contact of the stop solution, which consists of acid, with the skin. It causes burns on contact. Work with protective gloves and goggles. If contact occurs, the burned area must be immediately and thoroughly rinsed with plenty of water. If necessary, a doctor should be consulted.
- Adherence to the prescribed protocol for performing the test is essential. ALPCO assumes no liability for any damage caused by unauthorized changes in the test procedure.
- The guidelines for carrying out quality control in testing laboratories must be observed. Appropriate controls must be tested.
- The reagents must not be used after the expiration date.
- Wear disposable gloves when handling samples or kit reagents and wash hands thoroughly afterwards. Do not pipette by mouth. Do not eat, drink, smoke, or apply makeup in areas where samples or kit reagents are handled.

- Samples may contain unknown interfering substances. This can lead to false high or false low results.

REAGENT PREPARATION

Microtiter plate. Take the required number of strips and assemble them in the holder. Ensure that the plate has reached 20-30°C before use. Store strips which are not needed at 2-8°C in the pouch with desiccant. Do not dispose of the holder until all strips are used.

Wash buffer. Dilute the wash buffer concentrate 1:10 with distilled or deionized water (1 part buffer + 9 parts DI water). When storing the wash buffer concentrate at 2-8 °C crystallization may occur. Before dilution, all crystals must be dissolved. The 1X working Wash Buffer is stable for 14 days at 2-8°C. It is recommended to dilute only the amount of buffer which is needed to process the given samples.

Standards and **Controls**: The standards and controls are ready to use, and are stable at -16°C up to the expiry date given on the label. A small volume (~0.5 µl) of the 1:10 1x working wash buffer should be reserved and used as **Standard 0** (please, refer to step 2 of "Sample preparation" for the amount to be pipetted). If running a partial plate, re-freeze remaining standards and controls immediately for one additional use. Do not exceed one freeze-thaw cycle.

All other test reagents are stable at 2-8 °C up to the date of expiry stated on the label, unless otherwise specified.

SAMPLE PREPARATION

Stool samples must be extracted using the Sample Buffer at a ratio of 1:50 before testing in the Anti-Gliadin sIgA/IgA ELISA. Manual weighing or an approved stool extraction device (80-EXDEV2-100) may be used.

For manual weighing, mix **15 mg** stool with **0.75 ml** Sample Buffer (or greater amount of stool diluted 1:50 with Sample Buffer). If using an approved extraction device (80-EXDEV2-100), add **0.75 ml** Sample Buffer then proceed with device instructions. Vortex until the mixture is homogenous. Transfer the resulting slurry to a plastic vial and centrifuge for 10 min at 3000 x g.

The supernatant is directly used in the Anti-Gliadin sIgA/IgA ELISA.

ASSAY PROCEDURE

All reagents and samples should be equilibrated to 20-30°C and mixed well before use.
The position of standards, controls, and samples are noted on a protocol sheet.

1. **Wash step**

   Pick out the pre-assembled microtiter plate with the needed number of strips and wash them 1x with 250 µl 1X working wash buffer. Remove residual buffer by tapping the plate on absorbent paper after the wash step.

2. **Sample incubation**

   Pipette **100 µl Standards, Controls, and stool extracts** in duplicate in the microtiter plate. For **standard 0**, use 100 µl of the 1X working wash buffer per well.

Cover the strips and incubate with shaking for **60 min** at room temperature (20-30 °C; 400 rpm, 2 mm orbit).

3. **Wash step**

   Discard the contents of the microwells and wash 5x with 250 µl 1X working wash buffer. Remove residual buffer by tapping the plate on absorbent paper after the last wash step.

4. **Conjugate incubation**

   Pipette **100 µl Conjugate** in each microwell.

   Cover the strips and incubate with shaking for **60 min** at room temperature (20-30°C; 400 rpm, 2 mm orbit).

5. **Wash step**

   Discard the contents of the microwells and wash 5x with 250 µl 1X working wash buffer. Remove residual buffer by tapping the plate on absorbent paper after the last wash step.

6. **Substrate incubation**

   Pipette **100 µl Substrate** in each microwell.

   Incubate for **10-15 min** at room temperature with shaking (20-30°C; 400 rpm, 2 mm orbit) in the dark.

7. **Stopping the reaction**

   Pipette **50 µl Stop Solution** in each microwell. Mix well.

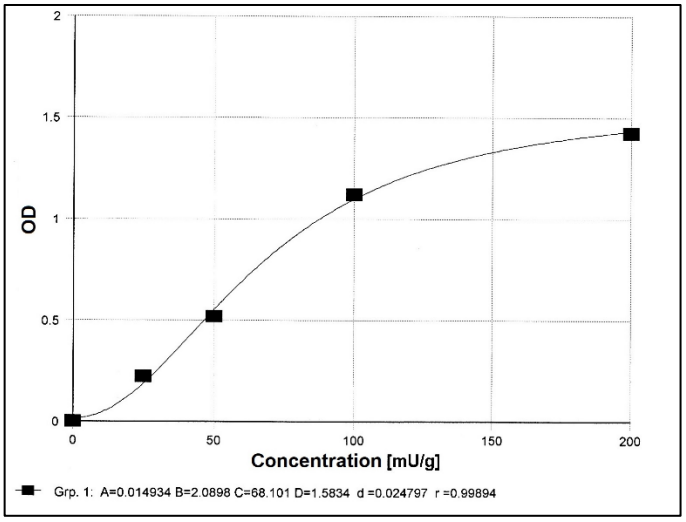
8. **Reading**

   Read the absorbance at 450 nm. If available, use 620 nm as reference wavelength. Read within **5 minutes** of stopping the reaction.

CALCULATION OF RESULTS

The use of the 4-parameter algorithm is recommended for calculation of results. The anti-gliadin sIgA/IgA concentration is read directly from the standard curve. (Listed standard values incorporate the 1:50 standard dilution.)

TYPICAL STANDARD CURVE



The curve at left is for demonstration purposes only. It must not be used for calculation of sample values.

PERFORMANCE CHARACTERISTICS

Measuring Range

The measuring range of the anti-gliadin sIgA/IgA assay is between a sample concentration of 25 mU/g and 200 mU/g.

Sensitivity

Limit of Detection: 2.1 mU/g

For the determination of the detection limit, 20 replicates of Standard 0 were measured. After addition of 3 x the standard deviation to the mean value of the optical density, the concentration was read from the standard curve.

Limit of Quantification: 4.6 mU/g

For the determination of the detection limit, 20 replicates of Standard 0 were measured. After addition of 10 x the standard deviation to the mean value of the optical density, the concentration was read from the standard curve.

Precision: Within run (intra-assay) variation

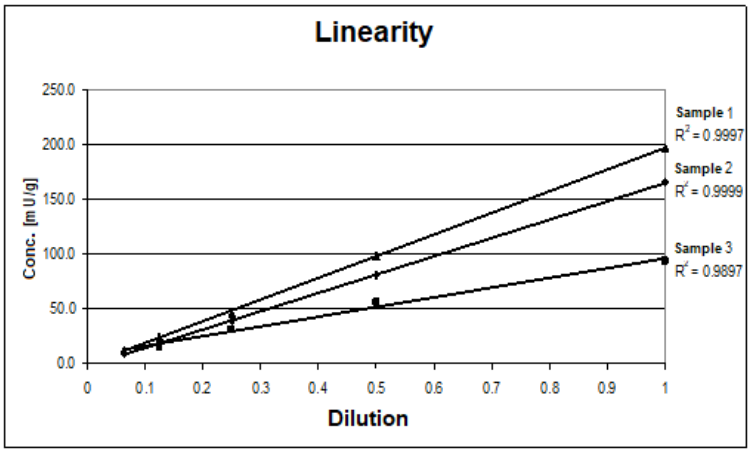
Intra-Assay CV Stool:	3.7 % (21.5 mU/g)	[n = 10]
	4.6 % (47.0 mU/g)	[n = 10]
	3.1 % (79.7 mU/g)	[n = 10]

Precision: Between run (inter-assay) variation

Inter-Assay CV Stool:	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

Dilution of the samples was performed with Sample Buffer



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

Spike and Recovery

Sample	Endogenous (µg/mL)	Added Conc. (µg/mL)	Expected (µg/mL)	Measured (µg/mL)	Recovery (%)
1	11.4	20 50 75	31.4 61.4 86.4	29.4 66.7 87.4	93.6 108.6 101.2
2	10.8	20 50 75	30.8 68.8 85.8	28.3 66.2 84.1	91.9 108.9 98.0

Cross-Reactivity

Cross reactivities could not be determined in an autoantibody test.

Limitations of the Method

Stool samples with anti-gliadin antibody concentrations above the standard curve should be diluted with sample buffer and measured again.

In the case of diarrhea, normal levels can be measured with increased antibody formation.

Disposal

The substrate must be disposed of as a non-halogenated solvent. The stop solution can be neutralized with NaOH and, if the pH value is neutral, it can be disposed of as a salt solution (**important:** this reaction produces heat and should be handled carefully). Please refer to local and national guidelines.

References

1. Balakireva, A.V., 2016, Properties of Gluten Intolerance: Gluten Structure, Evolution, Pathogenicity and Detoxification Capabilities**,** Nutrients, 8(10):644.
2. Barbaro, M.R., 2018, Recent advances in understanding non-celiac gluten sensitivity, F1000 Research, 7:1631.