Gliadin ELISA Kit

Enzyme Immunoassay for the quantification of Gliadin in food (extraction, dilution).

Catalog number: ARG80801

Package: 96 wells

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INTRODUCTION

Gliadin (a type of prolamin) is a class of proteins present in wheat and several other cereals within the grass genus *Triticum*. Gliadins, which are a component of gluten, are essential for giving bread the ability to rise properly during baking. Gliadins and glutenins are the two main components of the gluten fraction of the wheat seed. This gluten is found in products such as wheat flour. Gluten is split about evenly between the gliadins and glutenins, although there are variations found in different sources.

Gliadin is the water-insoluble component of gluten, and glutenin is water-soluble. There are three main types of gliadin (α, γ, and ω), to which the body is intolerant in coeliac (or celiac) disease. Diagnosis of this disease has recently been improving.

Gliadin can cross the intestinal epithelium. Breast milk of healthy human mothers who eat gluten-containing foods presents high levels of non-degraded gliadin. [Provide by Wikipedia: Gliadin]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A capture antibody specific for Gliadin (the soluble fraction of gluten) has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Gliadin present is bound by the immobilized antibody. After washing away any unbound substances, an HRP-conjugated antibody specific for Gliadin is added to each well and incubate. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of Gliadin bound in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of Gliadin in the samples is then determined by comparing the O.D of samples to the standard curve. Because of the equal amounts of gliadin and glutenin in wheat gluten, the gluten concentration of the sample is calculated by multiplication with the factor 2.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
Standard 1-5 (0, 2, 6, 20, 60 ppm Gliadin, dyed red)	2 mL each (ready to use)	4°C
Antibody Conjugate (Anti-Gliadin-Peroxidase, dyed red)	15 mL (ready to use)	4°C
10X Diluent Buffer (dyed red)	60 mL	4°C
10X Wash Buffer	60 mL	4°C
TMB substrate	15 mL (ready to use)	4°C (protect from light)
STOP solution	15 mL (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Ethanol (40%)
- Skimmed milk powder (used when indicate)
- Mixer or Ultra-Turrax
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)
- Plastic bag to store unused microplate strips

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
- Due to the high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be cleaned thoroughly before and after each sample. To identify possible cross contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer and 10X Diluent Buffer, warm to 37°C for 15 min or until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Use a new adhesive plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra

precaution to avoid plate strips from coming loose during the procedure.

- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates.

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Beer samples:

1. Degas beer, for instance by ultrasonics.
2. Dilute 100 µL of beer with 4.9 mL of 1X Diluent Buffer.
3. The samples are centrifuged for 10 minutes at 2500 g. The particle-free supernatant has to be applied in the test procedure.

Other kinds of samples: (extraction process with 40% ethanol is needed)

1. To maximize homogeneity and representativeness of the sample collection, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill etc.
2. 1 g of the homogenized mixture is suspended in 10 mL of 40% ethanol. If tannin-containing food like chocolate is extracted, 1 g of skimmed milk powder is added before suspension. Afterwards the suspension is mixed for further 5 min to ensure good homogeneity.
3. The samples are centrifuged for 10 minutes at 2500 g. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.
4. Afterwards the particle-free solution is diluted 1:50 in 1X Diluent Buffer (for example, 20 µL of solution in 980 µL 1X Diluent Buffer).

Note:

1. Samples containing sodium azide should not be used in the assay.
2. Collect samples and assay immediately, or aliquot and store at -80°C. Avoid repeated freeze-thaw cycles. Perform dilutions in 1X Diluent Buffer as necessary.
3. Due to the high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be cleaned thoroughly before and after each sample. To identify possible cross contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 10X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 50 ml of 10X Wash Buffer into 450 ml of distilled water to a final volume of 500 ml) The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C.
- 1X Diluent Buffer: Dilute 10X Diluent Buffer into distilled water to yield 1X Diluent Buffer. (E.g., add 50 ml of 10X Diluent Buffer into 450 ml of distilled water to a final volume of 500 ml) The 1X Diluent Buffer is stable for a least one week at 2-8°C.
- Sample: If the results of a sample are out of the measuring range, further dilution with the 1X Diluent Buffer is necessary. The additional dilution has to be considered when calculating the concentration.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

1. Add **100 µL** of **samples or Standards** to the Antibody-coated microplate.
2. Incubate at **RT** for **20 mins.**
3. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes.** Wash by filling each well with **1× Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of the **Antibody Conjugate** to each well. Incubate at **RT** for **20 mins** on a microplate shaker.
5. Aspirate each well and **wash as step 3**.
6. Warm **TMB substrate** to **RT**. Add **100 µL** of **TMB Substrate** to each well. Incubate for **20 minutes** at **room temperature** in dark.
7. Add **100 µL** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at **450nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance within 30 minutes after adding the stop solution.

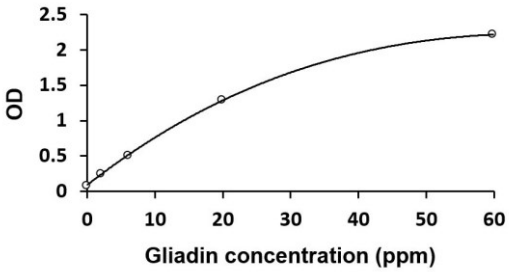
CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.
2. The ready-to-use standards are prepared for a direct determination of sample concentrations. The dilution of samples in the extraction process as described in the above stated sample preparation procedure is already considered.
3. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
4. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
5. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
6. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
7. The ready-to-use standards are prepared for a direct determination of sample concentrations. The dilution of samples in the extraction process as described in the above stated sample preparation procedure is already considered. In case of beer samples the resulting concentration has to be divided by 10.

8. If the samples have been further diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.
9. For calculating the corresponding gluten concentration, the result of gliadin has to be multiplicated with factor 2.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The limit of detection (LOD) of this kit is 0.03 ppm Gliadin for beer samples and 0.3 ppm Gliadin for all other samples.

The limit of quantification (LOQ) of this kit is 0.2 ppm Gliadin for beer samples and 2 ppm Gliadin for all other samples.

Intra-assay and Inter-assay precision

The CV value of intra-assay was 4-5% and inter-assay precision was 2-3%

Linearity

95% - 115%

Recovery

Mean recovery was determined by spiking samples with different amounts of gliadin:

Rice wafer	97%
Corn semolina	98%
Dark chocolate	97%
Baby food	88%
Sausage	85%
Beer	96%

Cross-reactivity

For the following foods no cross-reactivity could be detected:

Milk, Rice, Amaranth, Pork, Corn, Quinoa, Beef, Buckwheat, Teff, Egg, Millet, Cocoa and Soy.

The gluten concentration in various foods may vary significantly. Additionally the determined concentration of different cereals depends on the cross-reactivity of the prolamin towards the wheat-gliadin antibody. The following table will give guidelines for the cross-reactivity of different cereals.

Cereals	Cross-reactivity [%]
Wheat	100
Rye	100
Barley	5
Triticale	40
Oats	< 0.003