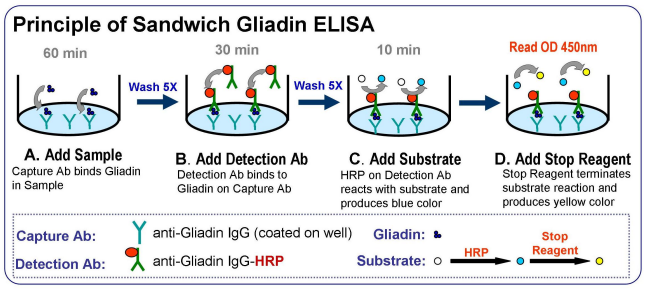
EnzyChrom™ Gliadin ELISA (EGLD-100)

Colorimetric Sandwich ELISA for Gliadin

DESCRIPTION

GLIADIN and glutenin are the two main proteins comprising gluten, a protein composite in the endosperm of grains. An intolerance to these gluten proteins results in celiac disease, a chronic autoimmune intestinal disorder characterized by bloating, diarrhea, and other abdominal pains. In addition to celiac disease, there are other forms of gluten intolerance that have led to the adoption of many gluten free alternatives in the food and beverage industry. Different countries have different restrictions regarding what foods can be labeled "gluten free" for gluten sensitive individuals. This has resulted in a need for accurate gluten quantification in food and beverage samples. Fortunately because the gliadin and glutenin fractions of gluten are almost evenly split, quantifying the total content of one of these proteins allows for accurate estimation of total gluten content in a sample.

BioAssay Systems' Gliadin ELISA uses a colorimetric sandwich ELISA method detailed in the figure below to accurately measure gliadin content in samples, which can then be used as an accurate estimate of total gluten content.



KEY FEATURES

Fast and Simple. Entire assay procedure takes less than three hours.

Sensitive and Accurate. Quantifies gliadin in the parts per billion range.

Simple Calculation. Simple calculation with linear standard curve.

Detection range: 0.1 – 2 ppb gliadin.

APPLICATIONS

Determination of gliadin in food and beverage samples.

KIT CONTENTS

10× Wash Buffer:	50 mL	**TMB Buffer:**	14 mL
5× Sample Buffer:	50 mL	**Stop Reagent:**	14 mL
TMB Substrate:	75 µL	**Detection Ab:**	75 µL
Standard (2,000 ppb):	50 µL	**Capture Stripwell Plate:**	1

Storage conditions: this kit is shipped on ice. Upon delivery, store Detection Ab and Standard at -20°C. Store all other reagents at 4°C. Shelf life of 12 months after receipt.

Note: all reagents may be stored at -20°C; however, precipitates may form in the 10× Wash Buffer and 5× Sample Buffer. If precipitates form, heat in a hot water bath until precipitates are solubilized before using.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

ASSAY PROCEDURE

Important:

1. To avoid cross-contamination, change pipette tips between additions of each reagent or sample. We recommend the use of a multi-channel pipette. Use separate reservoirs for each reagent. Prior to assay, dilute 10× Wash Buffer in dH_2O to prepare 500 mL 1× Wash Buffer. Dilute 5× Sample Buffer in dH_2O to prepare 250 mL 1× Sample Buffer. (*Note: If 5× Sample Buffer has precipitate, heat in a warm water bath until the precipitate is solubilized.*)
2. It is recommended that samples be assayed in duplicate or higher.
3. Please see "Materials Required But Not Provided" section for a list of additional reagents and equipment that are needed.

A. Sample Preparation

1. Thoroughly homogenize sample to a fine consistency in a clean, contamination free homogenizer. Samples that are already a fine consistency (e.g. powders, pastes, liquids) can skip this step.
2. Weigh out 1 g of sample in a clean, disposable 15 mL polypropylene centrifuge tube. For liquid samples, add 1mL of sample to a clean, disposable 15 mL polypropylene centrifuge tube.
3. Add 9 mL 40% Ethanol and vortex thoroughly for 1-2 minutes to homogenize the sample. This makes a 10% w/v solution for solid samples and a 10% v/v solution for liquid samples.
5. Tightly cap the tube and place in a 100°C water bath. Incubate for 15 minutes. Remove tube from heat source and rinse tube under cold water to cool to RT.
7. Vortex tube thoroughly 1–2 minutes to homogenize the sample. Aliquot 1 mL to a 1.5 mL centrifuge tube. For thick, viscous solutions you may wish to widen the pipette by cutting off a portion of the tip. Centrifuge for 10 minutes at 5,000 g. Collect the supernatant as the Sample Extract.
8. Dilute enough of the Sample Extract for the desired number of replicates in 1× Sample Buffer using the following dilution guidelines. For unknown samples, you may wish to run a serial dilution to determine the best dilution factor to use. *Note: be sure to factor in that the Sample Extract is a 10% w/v or v/v solution of the original sample when correcting for dilution factor in the calculation.*

Original Sample Gluten Content	Recommended Dilution of Sample Extract
Very High Gluten (1 – 20%)	100,000 – 1,000,000
Low Gluten (20 – 100 ppm)	1,000 – 10,000
Very Low Gluten (< 20 ppm)	100 – 1,000

B. Kit Reagents

1. Bring kit Reagents to RT. Remove capture stripwell plate from vacuum sealed bag and takeout required number of strips to accommodate all samples and standards to be run. Keep Detection Ab on ice or at 4°C when not in use. Return unused strips to bag and store at 4°C.

C. Standards

1. Prepare 500 µL 20 ppb standard: mix 5 µL 2,000 ppb Gliadin Standard with 495 µL 1× Sample Buffer.
2. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

Standard No.	Standard Concentration	Volume of 1× Sample Buffer	Volume of 20 ppb Standard
1	2.0 ppb	450 µL	50 µL
2	1.2 ppb	470 µL	30 µL
3	0.6 ppb	485 µL	15 µL
4	0 ppb	500 µL	0 µL

D. Gliadin Capture

1. Add 100 µL of Standards to wells in duplicate. Add 100 µL of appropriately diluted Samples to wells in desired number of replicates (e.g. duplicates, triplicates, quadruplicates, etc).
2. Incubate 1 hour at room temperature.
3. Aspirate wells and wash 5× with 300 µL 1× Wash Buffer.

E. Add Detection Antibody

1. For each 8-well strip prepare 1 mL of Detection Reagent as follows: 5 µL Detection Ab + 1mL 1× Wash Buffer.
2. Add 110 µL Detection Reagent to each well.
3. Incubate 30 min at room temperature.
4. Aspirate wells and wash 5× with 300 µL 1× Wash Buffer.

E. Detection

1. For each 8-well strip prepare 1 mL TMB Reagent as follows: 5 µL TMB Substrate + 1mL TMB Buffer.
2. Quickly add 100 µL TMB Reagent to each well.
3. Incubate plate 30 min protected from light.
4. Quickly add 100 µL Stop Reagent to each well.
5. Read OD at 450 nm.

CALCULATION

Subtract blank value (Standard #4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the gliadin concentration of Sample as follows:

$$[\text{Gliadin}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope}} \times n \ \text{(ppb)}$$

where OD_{SAMPLE} and OD_{BLANK} are optical density readings of the Sample and Blank (Standard #4), respectively. n is the sample dilution factor.

Note: if the sample OD value is higher than the OD for the 2.0 ppb gliadin standard, further dilute the sample in 1× Sample Buffer and repeat the assay.

Conversions:

1 mg/L = 1 ppm = 1,000 ppb;

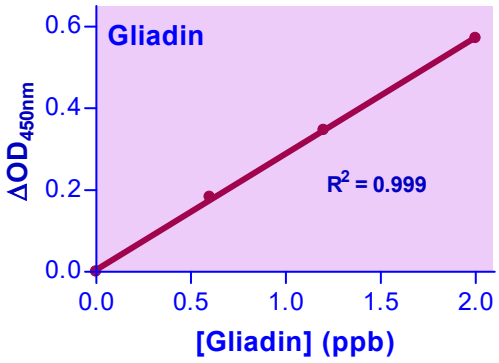
1 mg/mL = 1,000 ppm = 10^6 ppb;

1% = 1 g/dL = 10,000 ppm = 10^7 ppb

For total gluten content of the sample multiply gliadin content by two (gliadin and glutenin are present in gluten in approximately a 1:1 ratio).

MATERIALS REQUIRED BUT NOT PROVIDED

40% Ethanol; 15 mL centrifuge tubes; deionized or distilled water; pipetting devices; 1.5 mL centrifuge tubes; table centrifuge; 100°C water bath; colorimetric plate reader capable of reading at OD_{450nm}; graphing software.



Gliadin Standard Curve

LITERATURE

1. Dahesh M, et al (2014). Polymeric assembly of gluten proteins in an aqueous ethanol solvent. J Phys Chem B. 118(38):11065-76.
2. Gil-Humanes J, et al (2014). Reduced-Gliadin Wheat Bread: An Alternative to the Gluten-Free Diet for Consumers Suffering Gluten-Related Pathologies. PLoS One. 9(3):e90898.
3. Thompson T, Simpson S (2015). A comparison of gluten levels in labeled gluten-free and certified gluten-free foods sold in the United States. Eur J Clin Nutr. 69(2) :143-6.