APPLICATION NOTE

Quantifying gluten in beer using an ASBC-approved ELISA method

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Benefits

- **Confidently determine gluten levels with a quantitative method approved for use with beer and other food products**
- **Save time by automating ELISA wash steps with the MultiWash+ Microplate Washer**
- **Obtain results quickly with SoftMax Pro Software**

Introduction

Quantifying gluten in beer

With the recent rise in the prevalence of celiac disease, monitoring gluten levels in food and beverage has become increasingly important as more people strive to avoid gluten. FDA guidelines specify that foods labeled 'gluten-free,' 'no gluten,' 'free of gluten,' or 'without gluten' must contain less than 20 parts per million (ppm) of gluten. This is the limit of quantitation (LOQ) for currently accepted test methods.

Gluten occurs naturally in wheat, rye, barley, and crosses of these grains. It is a mixture of prolamin and glutelin proteins present in these grains. When foods are processed, and during digestion, intact prolamin is degraded into small peptide fragments which remain dangerous to celiac patients. These small peptide fragments cannot be detected by a sandwich ELISA due to that assay's requirement for two epitopes. However, the RIDASCREEN® Gliadin competitive ELISA can detect these small fragments using a new standard material, hydrolysate of wheat, rye, and barley (Figure 1). The R5 antibody used in this kit recognizes potentially toxic peptide sequences of gliadins from wheat and prolamins from rye and barley (Kahlenberg et al., 2005).

Many products are available for testing gluten content in a variety of sample types, but not all are quantitative, and not all are approved test methods. The RIDASCREEN® Gliadin competitive ELISA is an AACC International approved method (38-55.01) and an AOAC-approved Official Method of Analysis (First Action OMA 2015.05) that has been evaluated in an international study by 18 labs for use with beer, starch syrup, and sourdough. In a second international collaborative study by the American Society of Brewing Chemists (ASBC), the RIDASCREEN® Gliadin competitive ELISA was evaluated by 15 labs for five different beers, and this ELISA is now an ASBC international approved method (Beer-49).

Here we tested the gliadin levels in six commercially available beers to determine gluten levels with the RIDASCREEN Gliadin competitive ELISA. Beers tested included gluten-free, reduced gluten, weizenbier (wheat beer), and other varieties. The ELISA was read on the SpectraMax® ABS Plus Microplate Reader and results were analyzed using SoftMax® Pro Software. Quantitative results confirmed gluten levels below 20 ppm in the gluten-free and reduced-gluten beers.

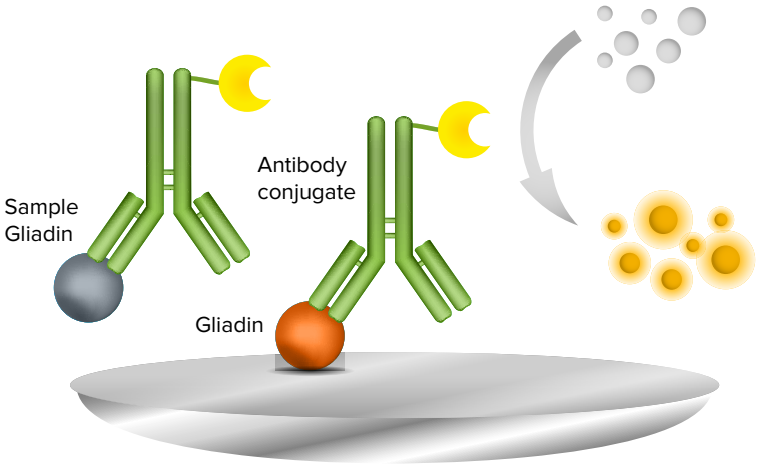

Figure 1. RIDASCREEN Gliadin competitive ELISA. Wells are precoated with gliadin. When sample and antibody conjugate are added, gluten in the sample competes for binding to the antibody, leading to a reduction in assay signal.

Materials

- RIDASCREEN Gliadin Competitive (Art. No. R7021)
- Commercially available beers:
  - Reduced gluten
  - Gluten-free
  - Weizenbier
  - Blond ale
  - American porter
  - India pale ale
- Cold water fish skin gelatin 40% (Electron Microscopy Sciences cat. #25560)
- Multi-Wash+™ Microplate Washer (Molecular Devices cat. #MultiWash+)
- SpectraMax ABS Plus Microplate Reader (Molecular Devices cat. #ABS Plus)

Reagent preparation

All reagents in the kit were prepared as indicated in the product insert.

- Buffer (sample diluent) was diluted 1:5 with deionized water.
- Wash buffer was warmed in a 37°C water bath to dissolve any crystals, and then diluted 1:10 with deionized water.
- Antibody enzyme conjugate was shaken before pipetting, then diluted 1:11 with deionized water. Only the amount needed for assay was diluted.
- Substrate/chromogen (Red Chromogen Pro) and Stop solution were supplied as ready-to-use solutions.

Beer sample preparation

1 mL of each beer was combined with 9 mL 60% ethanol solution containing 10% cold water fish skin gelatin in a 15-mL conical tube. Samples were mixed thoroughly by vortexing and then placed on a rotator for 10 minutes. Samples were then centrifuged at 2500 rpm for 10 minutes. Supernatants were then ready for assay and could also be stored in closed containers in the dark at room temperature for up to four weeks. Supernatants were diluted 1:50 with diluted sample diluent (buffer) prior to assay.

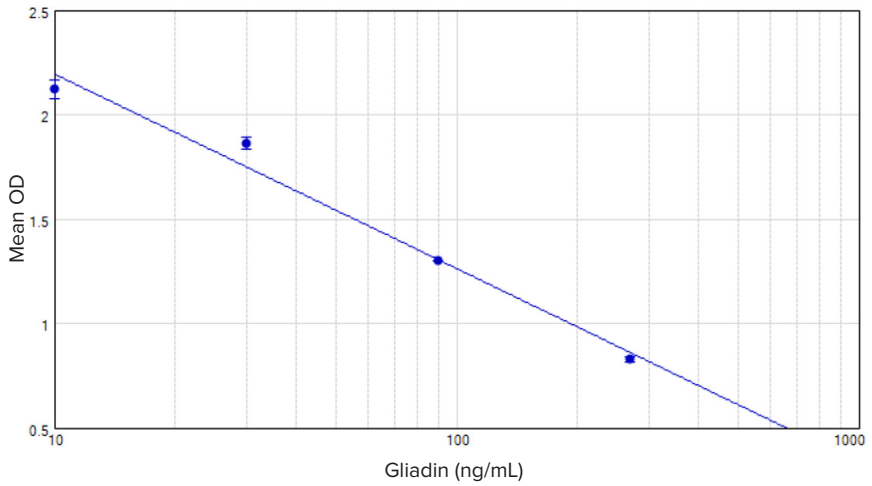

Figure 2. Gliadin ELISA standard curve. SoftMax Pro Software generated a standard curve including 10, 30, 90 and 270 ng/mL gliadin standards using a semi-log curve fit (R^2=0.981).

Sample	Wells	OD	R	Conc	AvgConc	SD	CV	Dilution Factor	AdjConc (ng/mL)	Gliadin (ppm)	Gluten (ppm)
01 Reduced Gluten	C2	2.061		13.922	16.763	4.0	24.0	500	8381.445	8.381	16.763
	D2	1.923		19.604							
02 Gluten Free	E2	2.098		12.717	9.032	5.2	57.7	500	4516.246	4.516	9.032
	F2	2.448	R	5.348							
03 Weizenbier	G2	0.486	R	683.442	706.316	32.3	4.6	500	353157.865	353.158	706.316
	H2	0.460	R	729.189							
04 Blond Ale	A3	1.298		91.844	87.242	6.5	7.5	500	43620.893	43.621	87.242
	B3	1.341		82.640							
05 American Porter	C3	1.230		108.663	118.153	13.4	11.4	500	59076.491	59.076	118.153
	D3	1.165		127.643							
06 India Pale Ale	E3	2.035		14.858	14.055	1.1	8.1	500	7027.733	7.028	14.055
	F3	2.081		13.253							

R - Outside standard range

Figure 3. Group table as seen in SoftMax Pro Software. Gliadin concentrations in beer samples were interpolated from the standard curve and multiplied by the dilution factor to obtain the adjusted concentration (ng/mL). Gliadin ppm and gluten ppm were calculated from the adjusted concentration.

ELISA

Three strip wells were placed into the microwell strip holder, and unused wells were stored in the foil pouch containing a desiccant packet.

50 µL of each gliadin standard (0, 10, 30, 90 and 270 ng/mL) or prepared sample was added to duplicate wells. Duplicate wells were also set aside as a plate blank (no standard or sample added). 50 µL of diluted antibody enzyme conjugate was added to each well and mixed manually by gently shaking the plate. The plate was incubated for 30 minutes at room temperature. Next, the plate was washed three times with 250 µL of diluted wash buffer using the MultiWash+ Microplate Washer.

100 µL of substrate/chromogen was added to each well, including plate blank wells. The plate was mixed gently by shaking manually, and incubated 10 minutes at room temperature in the dark. 100 µL stop solution was then added to every well, and the plate was mixed gently by shaking manually. Within 10 minutes, absorbance was measured at 450 nm on the SpectraMax ABS Plus reader using an ELISA protocol in the protocol library of SoftMax Pro Software.

A standard curve was plotted using the semi-log curve fit in SoftMax Pro Software, and sample concentrations were calculated from the standard curve.

Results

From the gliadin standard curve (Figure 2), gliadin concentrations were interpolated automatically by the software. Concentrations (ng/mL) were adjusted to the values present in the original beer samples by multiplying by the dilution factor (500), and gliadin (ng/mL) was converted to gliadin (ppm) by dividing by 1000. To convert gliadin into gluten, a factor of two was used (definition Codex Alimentarius), with results ultimately expressed as gluten ppm (Figure 3). These calculations were added to the 'Unknowns' group table of the protocol in SoftMax Pro Software.

Beers labeled reduced gluten or gluten-free had calculated gluten concentrations below 20 ppm, meeting FDA guidelines for these labels. For one of the gluten-free beer sample replicates, the OD value fell below that of the lowest gliadin standard, and this replicate was flagged as out of range, 'R' in the group table (Figure 3). Weizenbier, which was expected to have a higher gluten content, indeed showed gliadin values that exceeded levels present in the 270 ng/mL standard and was also flagged as out of range in the group table. To obtain an accurate quantitation of gluten, this beer would need to be further diluted. All the other beers tested ranged from 14 to 118 ppm gluten, with India pale ale falling beneath the 20 ppm 'gluten-free' level.

The blond ale, at 87 ppm gluten, could be labeled as 'very low gluten' by Codex Alimentarius standards.

Similar results are obtained using SpectraMax multi-mode readers (results not shown).

Conclusion

The RIDASCREEN Gliadin competitive ELISA provides a quantitative solution for gluten detection in beer. The hands-on time required to run this assay is reduced by using the MultiWash+ washer to automate wash steps. Detection of absorbance with the SpectraMax ABS Plus reader, and calculation of results using a SoftMax Pro Software protocol, further simplify the ELISA workflow and flag any result that warrants further attention. From sample to result, the time required to reliably determine gluten content is only about an hour.

Reference

Kahlenberg F., Sanchez D., Lachmann I., Tuckova L., Tlaskalova H., Mendez E., Mothes T. (2005). Monoclonal antibody R5 for detection of putatively coeliac-toxic gliadin peptides. *European Food Research and Technology* 222(1-2), 78-82.

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