

Accurate Detection of Gluten in Cake Mixes Using the Hygiena™ GlutenTox® Rapid G12 Kit

Introduction

The GlutenTox® ELISA Rapid G12 was developed to detect and accurately quantify gluten in food to ensure food safety for celiac patients that need to follow a life-long gluten-free diet. For this to be possible, it is important to have methods to measure gluten especially near and below the levels of 20 ppm which is the legal threshold in the EU and USA regulation (EU commission and FDA).

Description of the Kit

The GlutenTox® ELISA Rapid G12 Kit uses the G12 antibody to identify a 33-mer peptide segment of the gliadin protein, which is part of the gluten protein complex found in food products. This “33-mer” peptide is the most immunotoxic part of the gluten protein and induces celiac disease in susceptible people. The 33-mer peptide also shows high resistance to complete digestion by human digestive enzymes. Focusing on this peptide fragment provides more objective information to help people with celiac disease and gluten intolerance to avoid foods that contain gluten.

The GlutenTox ELISA Rapid G12 provides fast, convenient and accurate measurement of gluten down to 0.6 ppm (0.3 ppm of gliadin). The kit is based on the enzyme-linked immunoassay (ELISA) test, a proven laboratory method. This test uses the G12 antibody fixed to a testing surface (the “capture” antibody). This antibody binds specifically to certain peptides (in this case, the 33-mer). A second antibody, the “detection antibody,” is then introduced, labeled with an enzyme so it and its accompanying target protein can be measured. Standards are also included in the kit to help analyze and ensure performance results. Using this kit, accurate, analytic measurement of gluten can be obtained in 90 minutes.

Verifying Accuracy and Precision

One way to constantly ensure the accuracy and precision of any test kit, including the GlutenTox ELISA is to participate in proficiency testing schemes.

A world renowned accredited provider of proficiency testing schemes, Fapas®, has been using real world samples supporting food and water testing industries since 1990. Proficiency testing is an independent check of your laboratory procedures that provides you with a completely confidential assessment of your capabilities to achieve consistent, accurate results. Participation in proficiency testing schemes not only allows you to demonstrate the validity of your systems and the technical ability of your staff, but it also helps you gain and maintain ISO/17025 accreditation. Confidence in your laboratory equipment, methods, and staff provides your customers with the reassurance that you are delivering the quality results they require.

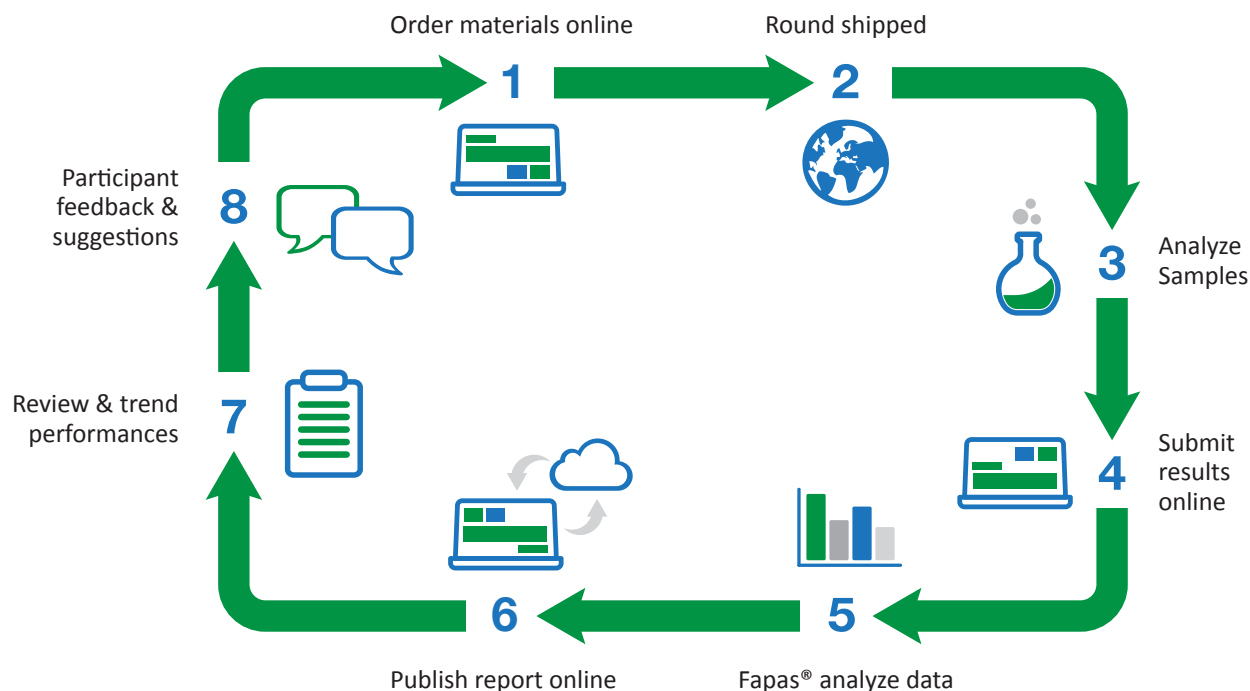
Proficiency testing is also a vital tool that can help to mitigate business risks such as product failure and non-compliance. Avoiding these risks can enhance your brand reputation, and help you meet legal requirements. Additionally, proficiency testing is one way of independently validating your laboratory results.

Gluten Testing

When it comes to gluten testing, regulations in the United States (US FDA) and European Union (EU No. 1169/2011 and Codex Alimentarius) stipulate that gluten-free products must have less than 20 ppm of gluten present. There are also regulations in Australia and New Zealand that mandate that gluten-free products have undetectable levels of gluten. Most often, immunological tests are done to detect gluten levels in food products. However, while these tests are sensitive, they often fail to accurately detect gluten levels in food products, especially those that are heavily processed.

In 2008, Codex Alimentarius endorsed Immunosorbent ELISA Assay methods using the Gluten R5 antibody as a Type 1 Method for gluten measurement in gluten-free foods.

The Fapas® Customer Journey



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In 2008, Codex Alimentarius endorsed Immunosorbent ELISA Assay methods using the Gluten R5 antibody as a Type 1 Method for gluten measurement in gluten-free foods.

Testing Method & Analysis

Currently, Fapas® proficiency-testing (PT) rounds are regularly performed in Europe and through representatives in the United States. To compare the performance of the Hygiena™ GlutenTox® ELISA Rapid G12 to other ELISAs, we requested Fapas PT samples for Gluten as part of the Fapas round 27275 – Gluten in Cake Mix. This proficiency study involved testing 2 cake mixes with unknown amounts of gluten (from relatively high amounts to relatively low amounts or none).

The sample provided were prepared from two different gluten, egg and dairy free cake mixes that were purchased from a retail source. Fapas then spiked these mixes with a combination of milk, egg, and gluten powders before tumble mixing them to produce a homogeneous test material.

Test material 27275A was spiked with 20 mg/kg of gluten powder; test material 27275B was spiked with 40 mg/kg of gluten powder. Samples were then sent to laboratories as PT samples for testing – labs had to report the values they obtained without knowing the actual spiked levels.

All labs testing the PT samples, including Hygiena™, had to indicate both qualitative and quantitative results of gluten presence and level as well as limit of detection and limit of quantification for all results provided back to Fapas. Fapas then summarized the data and provided a signed report to all participants with comparison to accepted standards for testing for gluten. Where possible, Fapas segregates the results into subsets according to the ELISA kit used.

As part of the analysis, data was statistically analyzed to provide an assigned value. The assigned values were used in combination with the standard deviation for proficiency to calculate a z-score for each result. The lower the z-score, the less variability in results; any $|z|$ value less than or equal to 2 was considered a 'passing' value.

Results

Fapas provided all lab participants with the testing results in a registered, official report. The Hygiena™ GlutenTox® ELISA Rapid G12 performed very well, detecting gluten in both test samples with accuracy similar to or better than that of other standard kits. In addition, when compared to the R-Biopharm Ridascreen kit, the GlutenTox® ELISA Rapid G12 kit showed tighter consistency of results. Comparative results are shown below.

Sample	Results with GlutenTox ELISA Rapid G12	Z-score	$-2 \leq z \leq 2$	Assigned value with Ridascreen Gliadin Kit (R7001)	'Theoretical' Fapas value (sample prepared with gluten of following value)
Cake mix A	13.11 ppm	-0.48	achieved	14.9 ppm	20 ppm
Cake mix B	30.53 ppm	-0.67	achieved	36.7 ppm	40 ppm

A collective summary of data from 6 other commercial kits is shown below (labeled as Figures 38 and 39 from the Fapas report) in dot plot format.

When the dot plot data is evaluated for all PT samples tested by all labs, the following data distribution is seen. Arrows represent the values obtained with the Hygiena™ GlutenTox® Rapid G12 ELISA kit. As you can see, GlutenTox results fell within the normal distribution of data, indicating that the Hygiena™ kit performs as well as the other tested kits.

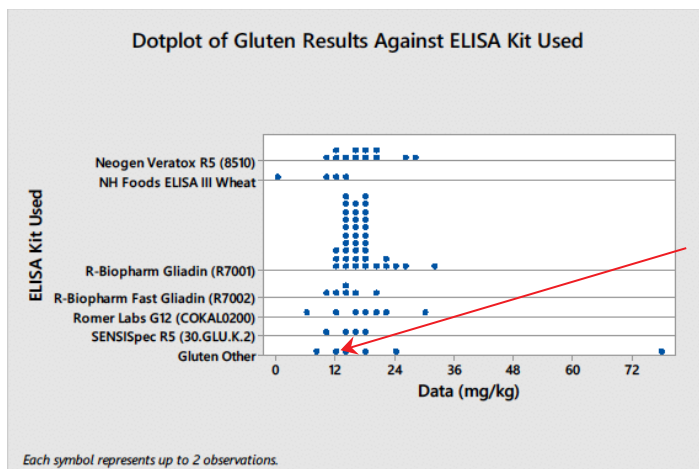


Figure 38: Dot plot of gluten in cake mix by kit type in 27275A

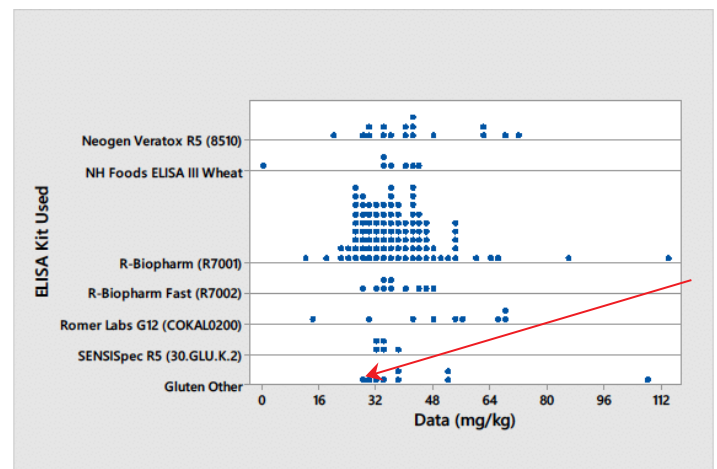


Figure 39: Dot plot of gluten in cake mix by kit type in 27275B

Conclusions

The Hygiena™ GlutenTox® Rapid G12 ELISA kit performed equally well to the commercial kits utilized in this PT study. Since the kit has a quantification range of 0.6 ppm to 200 ppm of gluten, and provides results in 90 minutes, it is an ideal choice for testing a wide range of food matrices for the presence of gluten at very low concentrations and also at higher concentrations. As the kit detects the immunotoxic fraction of gluten in food samples, it provides an accurate measure of the gluten present.

Appendix: Additional Analyses

Additional testing was completed with the GlutenTox ELISA Rapid G12. Results showed that the GlutenTox ELISA Rapid G12 can produce quantitative results of gluten concentration consistent with the assigned values from the Ridascreen Gliadin kit (table below) and from the most used ELISA kits (data not shown), according to historical Hygiena™ participation in other FAPAS proficiency testing rounds.

	Matrix	Assigned value from Ridascreen Gliadin Kit (R7001) (mg/kg)	Submitted values with GlutenTox ELISA Rapid G12 (mg/kg)		Sample was prepared to contain (mg/kg)
Material 27240B	Infant Soy Formula	37.1	32.4 (Z = -0.51)	33.51 (Z = -0.39)	35
Material 27247B	Cake Mix	19.3*	11.06 (Z = -1.71)	n/a	15
Material 27251A	Cake Mix	54.1	39.96 (Z = -1.05)	39.2 (Z = -1.10)	45
Material 27251B	Cake Mix	34.3	25.59 (Z = -1.02)	31.5 (Z = -0.33)	30
Material 27252A	Oat Based Foodstuff	16.6*	10.4 (Z = -1.49)	10.88 (Z = -1.38)	15
Material 27262	Cooked Biscuit	76.0	72.94 (Z = -0.16)	77 (Z = -0.05)	n/a
Material 27264B	Infant Soy Formula	24.8	28.3 (Z = 0.56)	27.2 (Z = 0.39)	28
Material 27271A	Cake Mix	39.2	24.8 (Z = -1.47)	n/a	35
Material 27276A	Oat Based Foodstuff	20.9	14.84 (Z = -1.16)	n/a	21
Material 27275A	Cake Mix	14.9	13.1 (Z = -0.48)	n/a	20
Material 27275B	Cake Mix	36.7	30.5 (Z = -0.68)	n/a	40

*These values represent a pool of the data of R7001 and R7002 (Gliadin Fast)