CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

051101

The AOAC Research Institute hereby certifies that the performance of the test kit known as:

EZ Gluten

manufactured by

ELISA Technologies, Inc.
2501 NW 66th Cr.
Gainesville, FL 32653
USA

This method has been evaluated in the AOAC® *Performance Tested Methods*SM Program, and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC® Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (September 27, 2018 – December 31, 2019). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

September 27, 2018
Date

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KIT NAME(S)
EZ Gluten

CATALOG NUMBERS
510EZG

INDEPENDENT LABORATORY
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APPLICABILITY OF METHOD
Target analyte – Gluten

Matrices – (0.5 g) - rice flour, cooked dough, beer, dog food, stainless steel (2 x 2 in)

Performance claims - This AOAC *Performance Tested Method*℠ study evaluated the EZ Gluten assay as an effective method for the detection of gluten in four selected matrices: rice flour, beer, cooked dough and dog food. In addition, the method was evaluated for its effectiveness in detecting gluten contamination of 1 mg or greater per 2 in^2 (25 cm^2) stainless steel surface area.

ORIGINAL CERTIFICATION DATE
May 31, 2011

CERTIFICATION RENEWAL RECORD
Renewed Annually through December 2019

METHOD MODIFICATION RECORD
NONE

SUMMARY OF MODIFICATION
NONE

Under this AOAC® *Performance Tested*℠ License Number, 051101 this method is distributed by:
NONE

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PRINCIPLE OF THE METHOD (1)
The EZ Gluten kit is a dipstick-style immunoassay that utilizes the anti-omega gliadin antibody developed by Skerritt and Hill[3]. This antibody, which reacts with both gliadins and glutenins, is the basis of the AOAC Official Method of Analysis for quantitative gluten analysis[4]. The EZ Gluten test has been optimized to allow this same antibody to detect gluten at levels as low as 10 parts per million (ppm). The EZ Gluten kit was designed for use by both consumers and industry to screen for the presence of gluten in foods and beverages.

DISCUSSION OF THE VALIDATION STUDY (1)
The EZ Gluten assay performed as expected in the selected matrices (rice flour, cooked dough, dog food and beer) and test conditions, meeting the product claim of detecting as little as 10 ppm of gluten. In testing three different spiking materials in four different matrices, the EZ Gluten demonstrated 100% specificity and 99% sensitivity at the 10 ppm level. The only false negative results at the 10 ppm level were seen with the bleached all-purpose flour material, and these did not appear to be matrix dependent.
The lot-to-lot data present evidence that the EZ Gluten assay is stable and can be consistently manufactured with reproducible quality. Robustness data indicated that the EZ Gluten can tolerate minor variations in protocol with the exception of the amount of time that the test strip is left in the sample extract. Due to the test format, there must be sufficient time for the sample extract to travel up the test strip, and this time cannot be shortened. This effect is greater when an insufficient sample size is used. A warning to this effect has been included in the test instructions.
Important comments were received by the independent laboratory which resulted in changes to the test instructions (Appendix A). The most valuable was the suggestion that "[a]dding a statement to the package kit insert instructing the user to interpret the [test] line as present regardless of how faint the line appears on the test strip may be beneficial to the user. It may also be helpful to include a statement in the package insert not to correlate the intensity of the hook or test line with the concentration of gluten. These additions to the package insert will add less subjectivity to the test." In response to this, the package insert now provides a method for the end user to increase the resolution of the bands on the test strip, instructions to interpret the presence of any pink line in the test area as positive, and a warning not to use band intensity to predict gluten concentration.
Changes to the final kit instructions for swabbing (Appendix B) were also made in response to the sensitivity of the test to 1µg of gluten contamination on a stainless steel surface.
The EZ Gluten assay can be recommended as a rapid qualitative screening assay for the presence of gluten in raw or cooked foods and beverages, and for the detection of gluten on environmental surfaces.

Table 1. Robustness Study Results (1)

Run Order	Sample Volume	Sample Settling Time	Extraction Volume	Strip Incubation Time	EZ Gluten Result for Each Replicate					POD	95% CI POD
1	0.4	4	8	5	N	N	N	N	N	0.0	0.00 - 0.43
2	0.6	4	12	20	P	P	P	P	P	1.0	0.57 - 1.00
3	0.4	6	8	5	N	N	P	N	N	0.2	0.04 - 0.62
4	0.4	6	8	20	P	P	P	P	P	1.0	0.57 - 1.00
5	0.4	6	12	5	N	N	N	N	N	0.0	0.00 - 0.43
6	0.6	6	8	5	P	N	N	P	P	0.6	0.23 - 0.88
7	0.4	4	12	5	N	N	N	P	N	0.2	0.04 - 0.62
8	0.4	4	8	20	P	P	P	P	P	1.0	0.57 - 1.00
9	0.6	6	12	5	P	P	P	P	N	0.8	0.38 - 0.96
10	0.6	4	8	20	P	P	P	P	P	1.0	0.57 - 1.00
11	0.6	6	8	20	P	P	P	P	P	1.0	0.57 - 1.00
12	0.6	4	8	5	P	N	N	P	N	0.4	0.12 - 0.77
13*	0.5	5	10	10	P	P	P	P	P	1.0	0.57 - 1.00
14	0.4	4	12	20	P	P	P	P	P	1.0	0.57 - 1.00
15	0.4	6	12	20	P	P	P	P	P	1.0	0.57 - 1.00
16	0.6	4	12	5	N	P	N	N	P	0.4	0.12 - 0.77
17	0.6	6	12	20	P	P	P	P	P	1.0	0.57 - 1.00

N = Negative, P = Positive, POD = Probability of Detection, CI = Confidence Interval
*Standard Conditions

Table 4. Commercial Flour Spikes in Rice Flour (1)

Spike Level (ppm)	Replicates (N)	Positives (x)	POD	95% CI POD
0	30	0	0.00	0.00 - 0.11
5	30	22	0.73	0.56 - 0.86
10	30	28	0.93	0.79 - 0.98
15	30	30	1.00	0.89 - 1.00
20	30	30	1.00	0.89 - 1.00

Table 5. NIST 1567a Wheat Flour Spikes in Rice Flour (1)

Spike Level (ppm)	Replicates (N)	Positives (x)	POD	95% CI POD
0	30	0	0.00	0.00 - 0.11
5	30	30	1.00	0.89 - 1.00
10	30	30	1.00	0.89 - 1.00
15	30	30	1.00	0.89 - 1.00
20	30	30	1.00	0.89 - 1.00

Table 6. PWG Gliadin Spikes in Rice Flour (1)

Spike Level (ppm)	Replicates (N)	Positives (x)	POD	95% CI POD
0	30	0	0.00	0.00 - 0.11
5	30	22	0.73	0.55 - 0.86
10	30	30	1.00	0.89 - 1.00
15	30	30	1.00	0.89 - 1.00
20	30	30	1.00	0.89 - 1.00

Table 7. Commercial Flour Spikes in Beer (1)

Spike Level (ppm)	Replicates (N)	Positives (x)	POD	95% CI POD
0	30	0	0.00	0.00 - 0.11
5	30	13	0.43	0.27 - 0.61
10	30	30	1.00	0.89 - 1.00
15	30	30	1.00	0.89 - 1.00
20	30	30	1.00	0.89 - 1.00

Table 8. NIST 1567a Wheat Flour Spikes in Beer (1)

Spike Level (ppm)	Replicates (N)	Positives (x)	POD	95% CI POD
0	30	0	0.00	0.00 - 0.11
5	30	30	1.00	0.89 - 1.00
10	30	30	1.00	0.89 - 1.00
15	30	30	1.00	0.89 - 1.00
20	30	30	1.00	0.89 - 1.00

Table 9. PWG Gliadin Spikes in Beer (1)

Spike Level (ppm)	Replicates (N)	Positives (x)	POD	95% CI POD
0	30	0	0.00	0.00 - 0.11
5	30	0	0.00	0.00 - 0.11
10	30	30	1.00	0.89 - 1.00
15	30	30	1.00	0.89 - 1.00
20	30	30	1.00	0.89 - 1.00

Table 10. Commercial Flour Spikes in Cooked Dough (1)

Spike Level (ppm)	Replicates (N)	Positives (x)	POD	95% CI POD
0	30	0	0.00	0.00 - 0.11
5	30	15	0.50	0.33 - 0.67
10	30	28	0.93	0.79 - 0.98
15	30	30	1.00	0.89 - 1.00
20	30	30	1.00	0.89 - 1.00

Table 11. NIST 1567a Wheat Flour Spikes in Cooked Dough (1)

Spike Level (ppm)	Replicates (N)	Positives (x)	POD	95% CI POD
0	30	0	0.00	0.00 - 0.11
5	30	30	1.00	0.89 - 1.00
10	30	30	1.00	0.89 - 1.00
15	30	30	1.00	0.89 - 1.00
20	30	30	1.00	0.89 - 1.00

Table 12. PWG Gliadin Spikes in Cooked Dough (1)

Spike Level (ppm)	Replicates (N)	Positives (x)	POD	95% CI POD
0	30	0	0.00	0.00 - 0.11
5	30	30	1.00	0.89 - 1.00
10	30	30	1.00	0.89 - 1.00
15	30	30	1.00	0.89 - 1.00
20	30	30	1.00	0.89 - 1.00

Table 13. Commercial Flour Spikes in Dog Food (1)

Spike Level (ppm)	Replicates (N)	Positives (x)	POD	95% CI POD
0	30	0	0.00	0.00 - 0.11
5	30	28	0.93	0.79 - 0.98
10	30	30	1.00	0.89 - 1.00
15	30	30	1.00	0.89 - 1.00
20	30	30	1.00	0.89 - 1.00

Table 14. NIST 1567a Wheat Flour Spikes in Dog Food (1)

Spike Level (ppm)	Replicates (N)	Positives (x)	POD	95% CI POD
0	30	0	0.00	0.00 - 0.11
5	30	30	1.00	0.89 - 1.00
10	30	30	1.00	0.89 - 1.00
15	30	30	1.00	0.89 - 1.00
20	30	30	1.00	0.89 - 1.00

Table 15. PWG Gliadin Spikes in Dog Food (1)

Spike Level (ppm)	Replicates (N)	Positives (x)	POD	95% CI POD
0	30	0	0.00	0.00 - 0.11
5	30	30	1.00	0.89 - 1.00
10	30	30	1.00	0.89 - 1.00
15	30	30	1.00	0.89 - 1.00
20	30	30	1.00	0.89 - 1.00

Table 16. Commercial Flour Contamination on Stainless Steel (1)

Spike Level (□g)	Replicates (N)	Positives (x)	POD	95% CI POD
0	30	1	0.03	0.01 - 0.17
1	30	30	1.00	0.89 - 1.00
10	30	30	1.00	0.89 - 1.00
50	30	30	1.00	0.89 - 1.00
100	30	30	1.00	0.89 - 1.00

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