VALIDATION REPORT
EZ GLUTEN® LATERAL FLOW ASSAY
AOAC Performance Tested Method℠ #051101
CATALOG NUMBER 510EZG
10 ppm Limit of Detection

Method Validation Summary
The following data represent the work carried out to validate the ELISA Technologies EZ Gluten® lateral flow assay following initial development. These data, along with the results of the AOAC PTM℠ validation, indicate that the method meets its product insert claims.

I. Reproducibility/Repeatability

Ia. Intra-Assay
This section is not applicable as these are single-use, single-sample tests.

Ib. Inter-Assay
Inter-assay repeatability was measured by having three operators run three replicates of a negative sample, a 10 ppm sample and a 20 ppm sample. The results are summarized below:

	0 ppm			10 ppm			20 ppm		
Replicate:	1	2	3	1	2	3	1	2	3
Operator									
1	N	N	N	P	P	P	P	P	P
2	N	N	N	P	P	P	P	P	P
3	N	N	N	P	P	P	P	P	P

N = visual negative result
P = visual positive result

Discussion
Sensitivity and specificity were both 100% in this multi-operator comparison.

II. Assay Drift
Assay drift was measured by testing a negative, 10 ppm, and 20 ppm sample with either the 10 minute standard incubation time, or with incubation times that were 5 minutes shorter or 5 minutes longer. Each sample was run in triplicate by one operator. The results are summarized on the following page:

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	0 ppm			10 ppm			20 ppm		
Replicate:	1	2	3	1	2	3	1	2	3
Incubation Time									
5 minutes	N	N	N	P	P	P	P	P	P
10 minutes*	N	N	N	P	P	P	P	P	P
20 minutes	N	N	N	P	P	P	P	P	P

*Standard incubation time
N= visual negative result
P = visual positive result

Discussion

Although a five minute incubation time produced valid results, this short incubation does not allow for sufficient migration along the test strip to fully clear the background. This background signal might cause problems in test interpretation. Therefore, using a shorter than recommended incubation time is to be avoided. Extending the incubation time to 20 minutes resulted in valid tests with clear backgrounds, indicating that over-incubation up to 20 minutes is not a concern.

III. Cross-reactivity/Interference

More than one hundred samples of unknown gluten content were tested using the EZ Gluten® assay. The results are listed below:

ETI ID	Sample	EZ Gluten® Result
V2	Strawberry-banana applesauce	N
V4	Butter beans	N
V5	French onion soup	N
V6	Tomato basil soup	P
V7	Spice mix	P
V9	Chicken canned cat food	P
V10	Vegetarian burger	P
V11	Stuffed pepper, frozen	N
V12	Beef gravy, jarred	P
V15	Corn soufflé, frozen	P
V17	Cherry danish	P
V18	Trail mix	P
V19	Pork roast, seasoned, raw	N
V21	Parmesan beef meat balls, raw	P
V22	Mayonnaise	N
V23	Pork florentine, raw	P
V24	Quiche, frozen	P
V25	Crab cakes, cooked	P
V26	Ground chicken, raw	N
V27	Chicken florentine, raw	P
V29	Popcorn chicken, fried	P
V31	Broccoli soup	N

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ETI ID	Sample	EZ Gluten® Result
V32	Vegetable juice	N
V33	Bran cereal	P
V34	Kitten chow	P
V35	Whole grain pasta	P
V37	Ranch dressing	P
V38	Lump crab	N
V39	Breaded vegetables, frozen	P
V40	Shiraz	N
V41	Chocolate/vanilla soy pudding	N
V42	Chocolate pudding	N
V43	Pudding	N
V44	Skim milk	N
V46	Whole milk	N
V47	Chocolate soymilk	N
V48	Vanilla soymilk	N
V49	Half & half	N
V50	Raspberry wheat ale	P
V51	Wheat-free brown rice flour	N
V52	Yellow corn meal	N
V53	Spelt flour	P
V54	Rye flour	P
V55	Buckwheat flour	P
V56	Soy flour	P
V57	Millet flour	N
V58	Barley flour	P
V67	Pumpernickel bread	P
V68	Cream of wheat	P
V69	Rice cakes	N
V70	Rye bread	P
V71	Gluten-free biscotti	N
V72	Chocolate grahams	P
V73	Baking chocolate	N
V74	Nutrition bar	N
V75	Milk chocolate	N
V76	White chocolate	N
V77	Cocoa powder	N
V78	Fat free yogurt	N
V79	Low-fat (1%) milk	N
V80	Coarse-ground mustard	P
V81	Greek yogurt	N
V83	Chocolate frosting	N
V84	Whole oats	N
V85	Toothpaste	N
V86	Whitening toothpaste	N
V87	Gluten-free breakfast bar	N
V88	Wheat-free millet rice cereal	P
V89	Gluten-free sweetened rice flakes	N
V90	Amaranth flakes	P

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ETI ID	Sample	EZ Gluten® Result
V91	Gluten-free arrowroot cookies	P
V92	Gluten-free pretzels	N
V93	Gluten-free baking mix	P
V94	Gluten-free crackers	N
V95	Gluten-free musli	N
V96	Gluten-free chocolate chip cookie mix	N
V97	Gluten-free pancake and waffle mix	N
V98	Gluten-free brown rice pasta	N
V99	Gluten-free quinoa flakes	N
V100	Wheat-free 7 grain cereal	P
V101	Gluten-free cream of buckwheat	N
V102	Gluten-free yellow corn grits	N
V103	Gluten free cookie and cake mix	N
V104	Gluten-free pancake and waffle mix	N
V105	Gluten-free bread crumbs	N
V106	Kamut	P
V107	Barley, hulled	P
V108	Sesame, hulled	N
V109	Basmati white rice	N
V110	Brown rice	N
V111	Kasha (buckwheat groats)	N
V112	Pumpkin seeds	N
V113	Rice blend	N
V114	Jasmine rice	N
V115	Gluten-free brown rice pasta	N
V116	Gluten-free pecan cookies	P
V117	Gluten free chocolate cake mix	P
V118	Gluten-free pancake mix	P
V119	Glazed Balsamic Vinegar	N
V120	Balsamic Vinegar	N
V121	Brown Rice Vinegar	N
V122	Tarragon Vinegar	N
V123	Ume Plum vinegar	N
CC	Dark chocolate	N
CC20	Dark chocolate spiked w/20 ppm gluten	P
MP	Milk Powder	N
LFMP	Lactose Free Milk Powder	N

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Additionally various oils were tested separately as a unique matrix to determine effects on extraction and possible interference of the assay. Oils were tested before (-) and after (+) being spiked with 10 ppm gluten (from an incurred wheat control) and extracted as liquid samples. Results are listed below.

Sample	EZ Gluten® Result
Negative Wheat Control	N
Positive Wheat Control	P
Vegetable Oil (-)	N
Vegetable Oil (+)	P
Cod Liver Oil (-)	N
Cod Liver Oil (+)	P
Extra Virgin Olive Oil (-)	N
Extra Virgin Olive Oil (+)	P
Sunflower Oil (-)	N
Sunflower Oil (+)	P
Vegetable Oil Spray (-)	N
Vegetable Oil Spray (+)	P
Margarine (-)	N
Margarine (+)	P
Butter – salted (-)	N
Butter – salted (+)	P
Vegetable Shortening (-)	N
Vegetable Shortening (+)	P
Lard (-)	N
Lard (+)	P
Flax Seed Oil (-)	N
Flax Seed Oil (+)	P

Discussion
The first 98 samples and oils were also tested using a quantitative ELISA method and demonstrated 100% agreement. Test results were also in agreement with stated ingredients on product labels, except in the case of gluten-free items which were found to be positive with the EZ Gluten® and confirmed by quantitative ELISA.

IV. Limit of Detection

Based on the results of sections I (Reproducibility/Repeatability) and II (Assay Drift), the limit of detection for wheat and rye is 10 ppm. The Skerritt antibody has been reported to be less sensitive to barley than to wheat or rye, so a separate set of tests was performed to determine the LOD for barley. One operator performed the EZ Gluten® assay at a range of barley concentrations. The results are summarized below:

Concentration	0 ppm	5 ppm	10 ppm	20 ppm	50 ppm	100 ppm	200 ppm
Result	N	N	P	P	P	P	P

N = visual negative result
P = visual positive result

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Discussion
Based on the above results, the limit of detection for wheat, rye, and barley is declared as 10 ppm.

V. Sample Stability

Sample extracts were stored at room temperature for 0, 3, or 7 days, then run in triplicate by one operator. The results are summarized below:

Time:	0 day @ RT			3 days @ RT			7 days @ RT		
	1	2	3	1	2	3	1	2	3
0 ppm	N	N	N	N	N	N	N	N	N
10 ppm	P	P	P	P	P	P	P	P	P
20 ppm	P	P	P	P	P	P	P	P	P

Discussion
Although sample extracts are stable following storage at room temperature for seven days, the product insert recommends that the extracts be used the same day.

VI. Test Protocol Variation

The only varied component of the assay is the incubation time, covered under section II (Assay Drift). See the AOAC PTM^{SM} validation for variations in sample size and extract volume used in testing.

VII. High Dose "Hook" Effect

No hook effect (loss of test line) was observed in samples with as much as 500 ppm of gluten. In our studies, the hook line begins to fade at levels greater than 200 ppm, and the test line remains visible at levels up to 10,000 ppm, confirming that this assay may be used reliably even at high gluten concentrations.

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FIT FOR USE CERTIFICATE

July 10, 2025

To Whom It May Concern:

This is to certify that the following immunoassay:

EZ GLUTEN® ASSAY

Produced by ELISA Technologies, Inc. at 2501 NW 66th Ct., Gainesville, FL 32653, has been thoroughly researched for performance in its intended use and for adherence to the product claims. Following review of the validation data, we are satisfied that this assay is fit for the use described in the instruction manual when the product is used as directed.

_____ 7/10/25

Justin Bickford, Scientific Director Date

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