CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

081202

The AOAC Research Institute hereby certifies the method known as:

Aller-Tek Gluten ELISA

manufactured by

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

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*[SM] Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[SM] certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date	November 26, 2023
Expiration Date	December 31, 2024

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MODIFICATION FEBRUARY 2018: ELISA Technologies, Inc.

SUBMITTING COMPANY
ELISA Technologies, Inc.
2501 NW 66th Ct.
Gainesville, FL32653

METHOD NAME
Aller-Tek Gluten ELISA

CATALOG NUMBER
510821

INDEPENDENT LABORATORY
Q Laboratories, Inc
1400 Harrison Avenue
Cincinnati, OH 45214 USA

APPLICABILITY OF METHOD
Target analyte – Gluten

Matrixes – (1 g) - rice flour (barley flour standard), rice flour (wheat flour standard), and cooked dough (wheat flour standard)

Performance claims - The Aller-Tek Gluten ELISA Assay was designed to quantitate low levels of gluten in food ingredients as well as in prepared and processed foods and beverages.

ORIGINAL CERTIFICATION DATE	**CERTIFICATION RENEWAL RECORD**
August 23, 2012	**Renewed Annually through December 2024.**

METHOD MODIFICATION RECORD	**SUMMARY OF MODIFICATION**
1. February 2018 Level 2	**1. Range limit change to add 2.5ppm and removal of 40ppm.**
2. September 2018 Level 1	**2. Editorial/Clerical changes, Trademark updates.**
3. September 2022 Level 1	**3. Editorial/clerical changes for clarity.**

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NONE	**NONE**

PRINCIPLE OF THE METHOD (1)
The Aller-Tek Gluten ELISA Assay uses a monoclonal antibody (401.21) which recognizes both the gliadin and glutenin fractions of gluten (5). This antibody is fixed to the wells of the 96-well plate and binds to available gluten when samples or standards are added to the well. Any unbound material is removed in the first wash step. The Gluten Conjugate, which is a solution containing the same 401.21 antibody bound to the horse radish peroxidase (HRP) enzyme, is then added and allowed to bind any gluten present in the wells. The second wash step removes any unbound gluten conjugate. Finally, a color substrate (TMB) is added, which causes a blue color change in proportion to the amount of HRP present in the well. The Stop Solution stops the TMB reaction and changes the blue color to yellow, and the intensity of this yellow color is then read on a plate reader.

DISCUSSION OF THE VALIDATION STUDY (1)
The AllerTek Gluten ELISA assay performed as expected for both wheat and barley flours in the selected matrixes (rice flour and cooked dough) and test conditions, meeting the product claims. Recovery at the regulatory level of 20 ppm averaged 118% for barley as well as for wheat in the combined in-house and independent data. The RSD_R for all samples 5 ppm or greater was 10.8%.
The lot-to-lot data present evidence that the AllerTek Gluten ELISA assay is stable and can be consistently manufactured with reproducible quality. Robustness data indicated that the AllerTek Gluten ELISA can tolerate minor variations in protocol, including wash method and extract settling time, but sample volume and particularly reagent volume are crucial to the precision of the test. Decreasing the reagent volumes by half resulted in significantly lower test results and may cause false negative test results in real-world testing.
Important comments were received by the independent laboratory which resulted in changes to the test instructions. This included a more specific description of the data analysis and a listing of the particular types of graphs that should be used in Excel for plotting the standard curve.
The AllerTek Gluten ELISA assay can be recommended as a quantitative screening assay for the presence of gluten in raw or cooked foods, including gluten from barley sources. Barley gluten can be accurately quantitated using a separate barley standard curve which is supplied on request with the test kit.

Table 2. ANOVA Analysis of Robustness Data (1)

ANOVA: AllerTek Gluten – Robustness					
Dependent variable: Result (ppm gluten)					
Independent variables: Wash method, Sample volume, Reagent volume Sample settling time					
Parameter	*Estimate*	*Standard Error*	*T Statistic*	*P-Value*	
CONSTANT	1.57118	0.261394	6.01077	0.0000	
Wash method	-0.236111	0.16025	-1.4734	0.1453	
Sample volume	0.0119549	0.00112637	10.6136	0.0000	
Reagent volume	0.0234132	0.00112637	20.7864	0.0000	
Settling time	0.00554398	0.00375457	1.4766	0.1445	
Analysis of Variance					
Source	*Sum of Squares*	*d.f.*	*Mean Square*	*F-Ratio*	*P-Value*
Model	256.889	4	64.2222	138.94	0.0000
Residual	30.97	67	0.462239		
Total (Corr.)	287.859	71			
R-squared = 89.2412 percent					
R-squared (adjusted for d.f.) = 88.5989 percent					
Standard Error of Est. = 0.679882					
Mean absolute error = 0.485817					
Durbin-Watson statistic = 1.24617 (P= 0.0001)					
Lag 1 residual autocorrelation = 0.360455					

Table 5. Wheat Flour in Rice Flour spike results

Raw Data						
	Replicates					
Spike Level	1	2	3	4	5	Average
0 ppm	0.2	0.5	0.3	0.4	0.1	0.3
5 ppm	5.2	4.9	6.2	5.7	6.3	5.7
10 ppm	10.1	11.2	12.0	12.4	12.9	11.7
20 ppm	24.3	19.4	22.0	20.6	20.8	21.4
40 ppm	40.1	49.9	55.2	44.4	50.1	47.9
80 ppm	85.7	90.1	81.0	83.7	75.6	83.2
Statistical Analysis						
Spike Level	s_r	RSD_r (%)	Bias	Recovery (%)	95% CI	
0 ppm	0.158				0.10	- 0.50
5 ppm	0.610	10.7	+0.7	114	4.90	- 6.42
10 ppm	1.098	9.4	+1.7	117	10.36	- 13.08
20 ppm	1.855	8.7	+1.4	107	19.12	- 23.72
40 ppm	5.814	12.1	+7.9	119	40.72	- 55.16
80 ppm	5.401	6.49	+3.2	104	76.51	- 89.93

Table 6. Wheat flour in cooked dough spike results (1)

Raw Data						
	Replicates					
Spike Level	1	2	3	4	5	Average
0 ppm	0.0	0.0	0.0	0.0	0.0	0.0
5 ppm	6.2	5.7	4.9	4.3	3.6	4.9
10 ppm	14	10.2	11.7	12.6	11.3	11.9
20 ppm	27.4	22.0	22.0	22.7	24.1	23.6
40 ppm	57.7	44.2	45.0	48.1	48.4	48.7
80 ppm	73.9	72.3	81.4	74.1	79.5	76.2
Statistical Analysis						
Spike Level	s_r	RSD_r (%)	Bias	Recovery (%)	95% CI	
0 ppm	0.000				0.00	- 0.00
5 ppm	1.045	21.3	-0.1	98	3.64	- 6.24
10 ppm	1.429	12.0	+1.9	119	10.19	- 13.73
20 ppm	2.270	9.6	+3.6	118	20.82	- 26.46
40 ppm	5.370	11.0	+8.7	121	42.01	- 55.35
80 ppm	3.963	5.2	-3.8	95	71.32	- 81.16

Table 7. Barley flour in rice flour spike results (1)

Raw Data						
	Replicates					
Spike Level	1	2	3	4	5	Average
0 ppm	1.4	1.4	1.3	1.4	1.4	1.4
5 ppm	5.1	5.8	4.9	4.9	5.4	5.2
10 ppm	7.4	7.7	8.4	7.6	8.0	7.8
20 ppm	27.0	24.3	23.1	21.3	22.6	23.7
40 ppm	44.5	45.8	42.1	41.2	42.5	43.2
80 ppm	88.8	85.2	88.5	91.2	88.8	88.5

Statistical Analysis						
Spike Level	s_r	RSD_r (%)	Bias	Recovery (%)	95% CI	
0 ppm	0.044				1.32	- 1.44
5 ppm	0.383	7.3	+0.2	104	4.74	- 5.70
10 ppm	0.389	4.9	-2.2	78	7.34	- 8.30
20 ppm	2.154	9.1	+3.7	118	20.98	- 26.34
40 ppm	1.880	4.4	+3.2	108	40.89	- 45.55
80 ppm	2.142	2.4	+8.5	111	85.84	- 91.16

REFERENCES CITED

1. Allred, Laura., Evaluation of the ELISA Technologies, Inc., Aller-Tek Gluten ELISA Assay for Qualitative Gluten Analysis, AOAC *Performance Tested Methods*^{SM} certification number 081201.
2. AOAC Research Institute Validation Outline for ELISA Technologies, Inc., Aller-Tek Gluten ELISA Assay for Qualitative Gluten Analysis, Approved – August 2012.