CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

011804

The AOAC Research Institute hereby certifies the method known as:

Wheat/Gluten ELISA Kit

manufactured by

Morinaga Bioscience, Inc.
2-1-1 Shimosueyoshi
Tsurumi-ku, Yokohama-shi, 230-8504
Japan

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*[SM] Program. 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.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date October 16, 2024
Expiration Date December 31, 2026

METHOD NAME	CATALOG NUMBER	ORIGINAL CERTIFICATION DATE
Wheat/Gluten ELISA Kit	M2103	January 29, 2018

PRINCIPLE OF THE METHOD

The Wheat/Gluten ELISA kit is a sandwich enzyme linked immunosorbent assay based on anti-gliadin polyclonal antibody. Gliadin is extracted from food samples by heating in boiling water for 10 minutes or shaking overnight with extraction buffer, which includes a detergent and reducing agent. A complex of antibody, antigen, and enzyme-conjugated antibody is formed by a two-step reaction and color development in the presence of enzyme with the addition of substrate. Absorbance is measured at a wavelength of 450 nm after the addition of a stop solution. The concentration of wheat protein/gluten, corresponding to the measured absorbance, can be determined by preparing a wheat protein standard solution curve.

CERTIFIED CLAIM STATEMENT: The Wheat/Gluten ELISA Kit method is certified for the quantification of wheat protein/gluten from wheat, barley, and rye cereal within the scope of Tables 1 and 2.

***Certification includes**:*

1. Bio-Rad Microplate Manager, software version 6.1.

Table 1. Method Performance Claims

Matrix	Test Portion	Solvent	Extraction and Assay type	Range, ppm	Performance supporting certification			
					LOD, mg/kg	LOQ, mg/kg	Recovery, %	RSD_r, %
Ice cream spiked with gluten	1 mL	Buffer[a]	Overnight and low range	0-14	0.04	0.13	76-82	4.0-8.2
			Short time and low range	0-14	0.03	0.09	76-83	4.1-6.5
			Overnight and high range	0-50	0.03	0.09	83-86	5.4-7.6
			Short time and high range	0-50	0.05	0.15	83-86	4.6-8.3
Water spiked with gluten	1 mL	Buffer	Overnight and low range	0-14	0.08	0.25	79-87	3.9-5.7
			Short time and low range	0-14	0.05	0.16	80-84	56.-6.6
			Overnight and high range	0-50	0.03	0.09	84-88	4.6-6.5
			Short time and high range	0-50	0.13	0.38	85-88	4.6-6.2
Water spiked with wheat protein	1 mL	Buffer	Overnight and low range	0-14	NR[b]	NR	99-115	5.0-8.0
			Short time and low range	0-14	NR	NR	93-120	6.5-8.4
			Overnight and high range	0-50	NR	NR	113-123	2.4-7.8
			Short time and high range	0-50	NR	NR	118-126	4.4-7.6
Soup	1 mL	Buffer	Overnight and low range	0-14	0.07	0.21	87-89	3.5-5.9

spiked with gluten			Short time and low range	0-14	0.05	0.15	82-88	4.8-7.8
			Overnight and high range	0-50	0.15	0.44	86-90	5.5-8.2
			Short time and high range	0-50	0.15	0.44	85-89	3.6-7.4
			Overnight and low range	0-14	0.03	0.08	93-121	4.6-12.0
Cider spiked with wheat protein	1 mL	Buffer	Short time and low range	0-14	0.02	0.06	93-118	5.8-8.2
			Overnight and high range	0-50	0.16	0.49	119-124	5.1-6.6
			Short time and high range	0-50	0.10	0.30	115-125	4.1-6.4
			Overnight and low range	0-14	0.05	0.14	111-129	3.2-10.1
Rice flour spiked with wheat protein	1 g	Buffer	Short time and low range	0-14	0.07	0.22	111-141	5.9-10.1
			Overnight and high range	0-50	0.14	0.41	125-136	4.5-8.1
			Short time and high range	0-50	0.07	0.22	137-144	5.0-8.2
			Overnight and low range	0-14	NR	NR	98.7-133.3	4.5-10.6
Gluten-free bread spiked with wheat protein	1 g	Buffer	Short time and low range	0-14	NR	NR	103.7-132.5	4.4-94
			Overnight and high range	0-50	NR	NR	112.7-127.7	5.6-7.9
			Short time and high range	0-50	NR	NR	111.8-133.2	4.2-8.2

[a] Proprietary formula.
[b] Not reported.

Table 2. Method Selectivity

Cross Reactivity			
	Commodities	Number of Positive Unspiked	
Gluten source	Tested	Overnight extraction + low range	Overnight extraction + high range
Gluten-free labeled products	38	1[a]	1[a]
Wheat	10	10	10
Rye	10	10	10
Barley	10	10	10

Interference				
	Spiked with 10 ppm gluten using PWG[b] Gliadin Spiking Solution		Spiked with 20 ppm gluten using PWG Gliadin Spiking Solution	
Gluten source	Overnight extraction + low range		Overnight extraction + high range	
	Recovery, %	RSD_r, %	Recovery, %	RSD_r, %
Wheat	132	3.8	121	3.8
Rye	114	7.3	109	5.5
Barley	70	9.1	67	3.1

[a] Oat flour was retested using 30 replicates. The results showed an average of 3.60 ppm with overnight extraction + low range assay and 9.61 ppm with overnight extraction + high range assay which is well below the gluten-free threshold of <20 ppm.
[b] Gliadin (87.7 % by weight) produced by the Prolamin Working Group.

Table 3. Method History

No.	Date	Summary	Supporting Data
1	January 2018	Original Certification.	Certification Report
2	September 2024	Modification – Manufacturing location change and company name change	Modification Report
3	October 2024	Company name change from "Morinaga Institute of Biological Science, Inc." to "Morinaga BioScience, Inc."	NA[a]

[a] Not applicable