



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
061201

The AOAC Research Institute hereby certifies the method known as:

Veratox[®] Gliadin R5 Test

manufactured by

Neogen Corporation
620 Lesher Place
Lansing, Michigan 48912
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.

A handwritten signature in black ink that reads "Scott Coates".

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date	December 27, 2023
Expiration Date	December 31, 2024

AUTHORS Anthony Lupo, Chris Roebuck, Aaron Walsh, and Mohamed Abouzied MODIFICATION AUGUST 2022: Marc Fowley, R. Lucas Gray, Brooke Roman, Robert Donofrio	SUBMITTING COMPANY Neogen Corporation 620 Leshler Place Lansing, Michigan 48912 USA
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METHOD NAME Veratox® Gliadin R5 Test	CATALOG NUMBER 8510
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INDEPENDENT LABORATORY
Food Allergen Research and Resource Program
University of Nebraska
Lincoln, Nebraska USA

APPLICABILITY OF METHOD Target organism – Prolamins from wheat (Gliadin), barley (hordein), and rye (secalin) Matrixes – Cooked hamburger (0.25g), bread (0.25g), rice breakfast cereal (0.25g), and rice flour (0.25g) Performance claims Sensitivity ≥ 97% Specificity ≥ 98% Agreement with FDA (BAM) culture method ≥ 94% Agreement with USDA FSIS culture method ≥ 97%	REFERENCE METHOD Official Methods of Analysis (2007) 18 th Ed., 2 nd Revision, 2007, AOAC INTERNATIONAL, Gaithersburg, MD, method 991.19 (2)
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ORIGINAL CERTIFICATION DATE June 11, 2012	CERTIFICATION RENEWAL RECORD Renewed annually through December 2024.
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METHOD MODIFICATION RECORD 1. December 2018 Level 1 2. November 2019 Level 1 3. August 2022 Level 2	SUMMARY OF MODIFICATION 1. Editorial/clerical changes. 2. Editorial/clerical changes. 3. Change in substrate composition for regulation and labelling requirements.
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Under this AOAC <i>Performance Tested Methods</i> SM License Number, 061201 this method is distributed by: NONE	Under this AOAC <i>Performance Tested Methods</i> SM License Number, 061201 this method is distributed as: NONE
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PRINCIPLE OF THE METHOD (1)

Veratox for Gliadin R5 is a sandwich enzyme-linked immunosorbent assay (ELISA). Gliadin is extracted from samples with an 80% ethanol solution by shaking in a shaker or rotator. Extract is diluted in phosphate saline buffer and diluted samples are added to R5 antibody-coated wells (capture antibody) where gliadin will bind to the antibody during an incubation period. Any unbound gliadin is washed away and a second antibody, which is R5 enzyme-labeled (detector antibody) is added. The detector antibody binds to the gliadin during another incubation period. Unbound enzyme-labeled antibody is washed away and a one step substrate is added. Color develops as a result of the presence of bound-labeled antibody. A stopping reagent is added and the color of the solution is observed. Blue color indicates samples containing high levels of gliadin while purple or red samples contain little or no gliadin. The optical densities of the controls form a standard curve, and the sample optical densities are plotted against the curve to calculate the exact concentration of gliadin in parts per million (ppm).

Table 2: Comparison to Existing Methods (1)

Samples	Mean Values (PPM)	
	Neogen	PTM 120601
Soy Flour	14.2	16.2
Rice Cereal	49.5	62.7
Rice Crisp	73.7	71.8
Granola Bars	33.5	43.4
Rice Cereal	57.8	64
Rice Mix	63.1	61.6
Soy Crisp	30	30.9
Ginger Cake Mix	46.6	48.6
Oat Cereal	29.6	29.1
Chocolate Wafers	40.3	40.1
Rice Cocoa Cereal	38.5	42.2
Multigrain Cereal	85.2	84
Corn Flakes Cereal	9	10.6
Chocolate Oat Cereal	14.1	15.3

All Results in ppm

REFERENCES CITED

1. Lupo, Anthony, Roebuck, Chris, Walsh, Aaron, and Abouzied, Mohamed., Validation of Rapid ELISA for Detection of Gliadin, AOAC Performance Tested MethodsSM certification number 061201.
2. Official Methods of Analysis (2007) 18th Ed., 2nd Revision, 2007, AOAC INTERNATIONAL, Gaithersburg, MD, method 991.19
3. Fowley, M., Gray, R.L., Roman, B., and Donofrio, R., Level 2 Modification Study to Validate Multiple Veratox® ELISA Methods, AOAC Performance Tested MethodsSM certification number 050901. Approved August 3, 2022.