CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

090901

The AOAC Research Institute hereby certifies the method known as:

Veratox® for DON 2/3 ELISA Deoxynivalenol

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.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

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

2275 Research Blvd., Ste. 300, Rockville, Maryland, USA Telephone: +1-301-924-7077 Fax: +1-301-924-7089
Internet e-mail: aoacri@aoac.org * World Wide Web Site: http://www.aoac.org

AUTHORS
ORIGINAL VALIDATION: Anthony Lupo, Chris Roebuck, Ken Settimo, Anna Quain, Justina Kennedy, and Mohamed Abouzied
MODIFICATION AUGUST 2022: Marc Fowley, R. Lucas Gray, Brooke Roman, Robert Donofrio

SUBMITTING COMPANY
Neogen Corp
620 Lesher Pl
Lansing, MI 48912 USA

METHOD NAME
Veratox® for DON 2/3 ELISA Deoxynivalenol

CATALOG NUMBER
8335

INDEPENDENT LABORATORY
U.S. Department of Agriculture Grain Inspection Packers and Stockyards Administration (GIPSA)
Technical Service Division
Kansas City, MO 64153 USA

APPLICABILITY OF METHOD
Target analyte – Mycotoxin Deoxynivalenol (DON).

Matrixes – Wheat, barley, malted barley, corn, oats, and rice

Performance claims
Precision – Less than 9.0% average CV for all levels tested.
Accuracy – 97% agreement with GC, with >95% DON recovery observed.
Cross-reactivity – With closely related compounds: 105% 3-acetyl DON, 7% 15-acetyl DON, and 3.8% nivalenol.
LOD – 0.1 ppm wheat, 0.1 ppm oats, and 0.1 ppm rice.
Range of quantitation.—The assay has a range of quantitation between 0.5 and 5.0 ppm without additional dilution, and range can be extended through further dilution with distilled water. Dilution in distilled water rather than uncontaminated sample matrix was chosen for ease of use and generated equivalent results.

REFERENCE METHOD

Tacke, B.K., & Casper, H.H. (1996) *J. AOAC Int.* **79**, 472–475. (6)

ORIGINAL CERTIFICATION DATE
September 2009

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2024.

METHOD MODIFICATION RECORD
1. **August 2022 Level 2**

SUMMARY OF MODIFICATION
1. **Change in substrate composition for regulation and labelling requirements.**

Under this AOAC *Performance Tested Methods*℠ License Number, 090901 this method is distributed by:
NONE

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NONE

PRINCIPLE OF THE METHOD (1)
Veratox for DON is a CD-ELISA in a microwell format that allows the user to obtain exact concentrations in parts per million (ppm). Free DON in the samples and controls is allowed to compete with enzyme-labeled DON (conjugate) for the antibody-binding sites. After a wash step, a substrate is added, which reacts with the bound conjugate to produce a blue color. More blue means less DON. The test is read in a microwell reader to yield optical densities. The optical densities of the controls form the standard curve, and the sample optical densities are plotted against the curve to calculate the quantitative results for DON levels.

DISCUSSION OF THE VALIDATION STUDY (1)
Given the observed accuracy over several levels of naturally contaminated reference material compared to GC and the degree of reproducibility observed in both the internal and collaborating laboratory data set, it is recommended that the Veratox for DON method be granted *Performance Tested Method* status.

Table 3. Direct comparison of GC to Veratox DON ELISA[a] (1)

GC	ELISA	Matrix
0.0	0.0	Corn
0.0	0.0	
0.3	0.3	
0.3	0.2	
0.6	0.6	
0.6	0.5	
1.1	1.0	
1.0	1.2	
2.3	2.0	
0.0	0.0	Wheat
0.0	0.0	
0.2	0.2	
0.5	0.4	
0.5	0.4	
1.1	1.2	
1.2	1.1	
2.2	1.9	
2.0	2.2	
2.3	2.2	Barley
0.0	0.0	
0.0	0.0	
0.3	0.3	
0.3	0.2	
0.5	0.5	
0.5	0.4	
0.9	1.0	
1.0	0.8	
2.1	1.8	
1.9	2.1	
1.9	1.8	
0.0	0.0	Malted barley
0.0	0.0	
0.3	0.3	
0.3	0.2	
0.5	0.6	
0.5	0.6	
1.1	1.2	
1.1	1.2	
2.2	1.9	

a n = 3 for each sample (averages shown). LOD for GC = 0.1 ppm. All results in ppm.

Table 5. Limit of detection using DON-free commodities[a] (1)

Replicate	Corn	Malted barley	Barley	Oats	Rice	Wheat
1	0.1	0.0	0.0	0.2	0.0	0.0
2	0.1	0.1	0.0	0.1	0.0	0.0
3	0.1	0.0	0.0	0.1	0.0	0.0
4	0.1	0.0	0.0	0.1	0.0	0.0
5	0.1	0.0	0.0	0.1	0.0	0.0
6	0.1	0.0	0.0	0.1	0.0	0.0
7	0.1	0.0	0.0	0.1	0.0	0.0
8	0.1	0.0	0.0	0.1	0.0	0.0
9	0.1	0.0	0.0	0.1	0.1	0.1
10	0.1	0.0	0.1	0.1	0.0	0.0
Mean	0.10	0.01	0.01	0.11	0.01	0.01
SD	0.00	0.03	0.03	0.01	0.03	0.03
Mean + 2SD	0.10	0.07	0.07	0.17	0.07	0.07

[a] LOD of GC = 0.1 ppm. All values in ppm. All samples contain no detectable amount of DON by GC.

Table 12. Results on fortified samples for additional commodities[a] (1)

	0.5 ppm					2.0 ppm				
Level	Corn	Malted barley	Barley	Oats	Rice	Corn	Malted barley	Barley	Oats	Rice
1	0.7	0.6	0.4	0.5	0.4	2.1	2.2	1.6	2.2	1.9
2	0.7	0.5	0.4	0.5	0.4	2.0	2.1	2.1	1.8	1.7
3	0.7	0.5	0.4	0.6	0.4	2.1	2.1	2.0	1.7	1.7
4	0.7	0.5	0.4	0.6	0.4	2.0	2.1	1.8	2.1	1.7
5	0.7	0.6	0.4	0.6	0.4	2.1	1.9	2.4	2.0	1.7
Mean	0.70	0.54	0.40	0.56	0.40	2.06	2.06	1.98	1.96	1.74
SD	0.00	0.06	0.00	0.06	0.00	0.06	0.11	0.30	0.21	0.09
%CV	0.0	11.1	0.0	10.7	0.0	2.9	5.3	15.2	10.7	5.2

[a] All results in ppm.

Table 13. Results of independent laboratory trials withthe Veratox DON ELISA[a,b] (1)

	Wheat, ppm		
	0.5	2.0	5.0
	Analyst A		
1	0.5	1.7	4.5
2	0.4	1.6	4.6
3	0.5	1.9	4.3
4	0.5	1.8	4.4
5	0.5	1.8	4.4
6	0.5	1.7	4.5
7	0.3	1.6	4.4
	Analyst B		
1	0.5	1.9	4.9
2	0.5	1.9	4.6
3	0.5	1.8	4.5
4	0.6	1.9	4.4
5	0.6	2.0	4.5
6	0.6	1.9	4.6
7	0.5	1.6	4.5
	Analyst C		
1	0.4	1.7	4.5
2	0.3	1.5	4.3
3	0.4	1.7	4.4
4	0.4	1.8	4.5
5	0.4	1.5	4.4
6	0.3	1.4	4.4
7	0.4	1.5	4.1
Mean	0.46	1.72	4.46
SD	0.09	0.17	0.15
%CV	20.25	9.69	3.43

[a] All results in ppm.

[b] Casper GC method: result for 0.5 ppm sample = 0.50 ppm; resultfor 2.0 ppm sample = 2.20 ppm; result for 5.0 ppm sample = 5.10 ppm.

REFERENCES CITED

1. Lupo, A., Roebuck, C., Settimo, K., Quain, A., Kennedy, J., and Abouzied, M., Validation Study of a Rapid ELISA for Detection of Deoxynivalenol in Wheat, Barely, Malted Barley, Corn, Oats, and Rice, AOAC Research Institute *Performance Tested Methods℠* certification number 090901.
2. Tacke, B.K., & Casper, H.H. (1996) *J. AOAC Int.* **79**, 472–475.
3. Fowley, M., Gray, R.L., Roman, B., and Donofrio, R., Level 2 Modification Study to Validate Multiple Veratox® ELISA Methods, AOAC *Performance Tested Methods℠* certification number 050901. Approved August 3, 2022.