



# CERTIFICATION

## AOAC Research Institute *Performance Tested Methods*<sup>SM</sup>

Certificate No.  
**071901**

The AOAC Research Institute hereby certifies the method known as:

### **Reveal® Q+ for DON Quantitative Test**

manufactured by

**Neogen**

**620 Leshar Place**

**Lansing, MI 48912**

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> 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*<sup>SM</sup> 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, appearing to read "Bradley A. Stawick".

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

Issue Date

January 15, 2024

Expiration Date

December 31, 2024

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<b>METHOD NAME</b> Reveal® Q+ for DON Quantitative Test	<b>CATALOG NUMBER</b> 8385
<b>INDEPENDENT LABORATORY</b> US Department of Agriculture Federal Grain Inspection Service 10383 N. Ambassador Dr. Kansas City, MO 64153 USA	<b>APPLICABILITY OF METHOD</b> <b>Analyte – Deoxynivalenol (DON)</b>  <b>Matrixes – corn, wheat, corn/soy blend, soybeans, barley, malted barley, buckwheat, brown rice, sorghum, distillers dried grain</b>  <b>Performance claims –</b> <b>LOD – corn, 0.014 ppm; wheat, 0.037 ppm</b> <b>LOQ – corn, 0.042 ppm; wheat 0.11 ppm</b> <b>Linearity – R<sup>2</sup> = 0.999</b> <b>Recovery – corn, 90-103%; wheat, 94-104%; other commodities 90-109%</b> <b>Range of quantitation 0.1 – 6 ppm. Range of quantitation with dilution 0.1 – 34.5 ppm.</b>
<b>ORIGINAL CERTIFICATION DATE</b> July 02, 2019	<b>CERTIFICATION RENEWAL RECORD</b> Renewed annually through December 2024.
<b>METHOD MODIFICATION RECORD</b> 1. November 2019 Level 1 2. December 2020 Level 1 3. November 2021 Level 1	<b>SUMMARY OF MODIFICATION</b> 1. Editorial changes. 2. Editorial changes, removal of ASIII information. 3. Insert formatting updated. Editorial changes to update FGIS method.
<b>Under this AOAC Performance Tested Methods<sup>SM</sup> License Number, 071901 this method is distributed by:</b> NONE	<b>Under this AOAC Performance Tested Methods<sup>SM</sup> License Number, 071901 this method is distributed as:</b> NONE

#### PRINCIPLE OF THE METHOD (1)

Reveal Q+ for DON is a single-step, lateral flow immunochromatographic assay for quantitative determination of deoxynivalenol (DON) in grain and grain products, including wheat, corn, barley, soybeans, buckwheat, rice, sorghum, and distillers dried grain. The test utilizes a competitive assay format. Sample extract is wicked through a reagent zone containing DON-specific antibodies conjugated to colloidal gold particles. The DON-antibody complex is then wicked onto a membrane containing a zone of DON conjugated to a carrier protein. Any uncomplexed antibody is captured in this zone, allowing the particles to concentrate and form a visible line. As the level of DON increases, less antibody-gold is captured in the test zone as more antibody-gold is bound to DON. Therefore, as the concentration of DON in the test sample increases, the test line density decreases. Algorithms programmed into the AccuScan® reader convert line density into a quantitative result displayed in parts per million (ppm). The test also contains a control zone and reagents utilizing a second immune complex. The control line will form regardless of the presence of DON in the test sample, ensuring that the test strip is functioning properly.

#### DISCUSSION OF THE VALIDATION STUDY (1)

Results of the study described here establish that the Reveal Q+ for DON test offers reliable performance for quantitative determination of DON in a wide variety of grain-based commodities. Recovery values were very close to 100%, ranging from 90 to 103% in corn, and from 94 to 104% in wheat. Mean recovery values across a range of DON concentration in eight additional commodities ranged from 90 to 109%. Linearity determination produced R<sup>2</sup> values of 0.999 in both corn and wheat. The LOD of the assay was established at 0.014 ppm in corn and 0.037 ppm in wheat. LOQ was determined to be 0.042 ppm in corn and 0.11 ppm in wheat. Results of independent laboratory testing using naturally contaminated corn and wheat supported those of internal testing.

Results of selectivity testing showed that the Reveal Q+ for DON test has no cross-reactivity with other mycotoxins or interference of these mycotoxins with the ability of the test to accurately determine DON concentration. The test does have some cross-reactivity with certain DON related compounds such as 3-acetyl-DON and DON 3-glucoside.

Results of experiments conducted to assess assay robustness showed that following the protocol as written is essential for accurate DON quantitation, with sample size and test strip development time being the most critical factors. Results of lot-to-lot consistency and stability studies showed consistency of results across a range of DON concentration and established that the Reveal Q+ DON reagents are stable for 18 months post-manufacture when stored under specified conditions.

The Reveal Q+ for DON test offers the advantages of aqueous sample extraction, minimal labor, hardware and bench space requirements, and just a 3-minute incubation.

**Table 2. Recoveries of DON from naturally contaminated corn and wheat (1)**

Matrix	Level (ppm)	N <sup>a</sup>	Mean (ppm)	S <sub>r</sub> <sup>b</sup>	RSD <sub>r</sub> (%) <sup>c</sup>	Recovery (%) <sup>d</sup>	Bias <sup>e</sup>
Corn	0.5	63	0.48	0.09	19.0	96	-0.02
	1.9	63	1.96	0.15	7.6	103	0.06
	4.8	63	4.52	0.17	3.8	94	-0.28
	9.7	63	9.87	0.69	7.0	102	0.17
	34.5	63	31.03	1.5	4.8	90	-3.47
	<i>Mean</i>					8.4	97
Corn <sup>f</sup>	0.5	21	0.44	0.05	12.4	88	-0.06
	2	21	1.88	0.09	4.9	94	-0.12
	30	21	30.48	1.56	5.1	102	0.48
	<i>Mean</i>				7.5	95	
Wheat	0.5	63	0.52	0.06	11.5	104	0.02
	2.1	63	2.17	0.20	9.2	103	0.07
	4.9	63	4.76	0.42	8.8	97	-0.14
	9.0	63	9.23	0.72	7.8	103	0.23
	28.9	63	27.11	1.9	7.0	94	-1.79
	<i>Mean</i>				8.9	100	
Wheat <sup>f</sup>	0.5	21	0.53	0.07	12.4	106	0.03
	2	21	2.30	0.10	4.5	115	0.30
	30	21	31.62	1.43	4.5	105	1.62
	<i>Mean</i>				7.1	109	

<sup>a</sup> N = Number of determinations<sup>b</sup> S<sub>r</sub> = Standard deviation<sup>c</sup> RSD<sub>r</sub> = Relative standard deviation (%)<sup>d</sup> Recovery = [mean<sub>cond</sub>/known concentration] x 100<sup>e</sup> Bias = mean<sub>cond</sub> – known concentration<sup>f</sup> Trial performed by independent laboratory**Table 3. Recoveries of DON from grains and grain products spiked with DON (1)**

Matrix	Level (ppm)	N <sup>a</sup>	Mean (ppm)	S <sub>r</sub> <sup>b</sup>	RSD <sub>r</sub> (%) <sup>c</sup>	Recovery (%) <sup>d</sup>	Bias <sup>e</sup>
Corn/soy blend	0.5	5	0.42	0.11	26.2	84	-0.08
	2.0	5	2.20	0.07	3.2	110	0.20
	5.0	5	4.92	0.18	3.6	98	-0.08
	30.0	5	29.12	0.91	3.1	97	-0.88
	<i>Mean</i>					9.0	97
Soybeans	0.5	5	0.40	0	0	80	-0.10
	2.0	5	2.06	0.09	4.4	103	0.06
	5.0	5	4.88	0.08	1.6	98	-0.12
	30.0	5	30.72	0.91	3.0	102	0.72
	<i>Mean</i>					2.2	96
Barley	0.5	5	0.40	0	0	80	-0.10
	2.0	5	1.80	0.07	3.9	90	-0.20
	5.0	5	4.82	0.04	0.8	96	-0.18
	30.0	5	30.08	0.91	3.0	100	0.08
	<i>Mean</i>					1.9	91
Malted barley	0.5	5	0.58	0.04	6.9	116	0.08
	2.0	5	2.44	0.09	3.7	122	0.44
	5.0	5	4.94	0.17	3.4	99	-0.06
	30.0	5	29.76	1.19	4.0	99	-0.24
	<i>Mean</i>				4.5	109	
Buckwheat	0.5	5	0.40	0	0	80	-0.10
	2.0	5	1.70	0.07	4.1	85	-0.30
	5.0	5	4.96	0.05	1.0	99	-0.04
	30.0	5	28.96	1.82	6.3	96	-1.04
	<i>Mean</i>				2.8	90	
Brown rice	0.5	5	0.30	0	0	60	-0.20
	2.0	5	2.02	0.04	2.0	101	0.02
	5.0	5	4.84	0.13	2.7	97	-0.16

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	30.0	5	30.72	0.44	1.4	102	0.72
	<i>Mean</i>				<i>1.5</i>	<i>90</i>	
Sorghum	0.5	5	0.48	0.04	8.3	96	-0.02
	2.0	5	2.00	0.07	3.5	100	0
	5.0	5	4.92	0.08	1.6	98	-0.08
	30.0	5	30.56	0.88	2.9	102	0.56
	<i>Mean</i>				<i>4.1</i>	<i>99</i>	
Distillers dried grain	0.5	5	0.60	0	0	120	0.10
	2.0	5	2.08	0.08	3.8	104	0.08
	5.0	5	4.70	0.10	2.1	94	-0.30
	30.0	5	25.92	0.44	1.7	86	-4.08
	<i>Mean</i>				<i>1.9</i>	<i>101</i>	

<sup>a</sup> N = Number of determinations

<sup>b</sup> S<sub>r</sub> = Standard deviation

<sup>c</sup> RSD<sub>r</sub> = Relative standard deviation (%)

<sup>d</sup> Recovery = [mean<sub>cand</sub>/known concentration] x 100

<sup>e</sup> Bias = mean<sub>cand</sub> – known concentration

**REFERENCES CITED**

1. Gray, R.L., Kostin, A., Anderson, G., Roman, B., Klein, F., and Donofrio, R., Determination of Deoxynivalenol in Grains and Grain Products Using the Reveal® Q+ for DON Method, AOAC Performance Tested Method<sup>SM</sup> certification number 071901.