



# CERTIFICATION

**AOAC<sup>®</sup> Performance Tested<sup>SM</sup>**

Certificate No.

**000701**

The AOAC Research Institute hereby certifies that the performance of the test kit known as:

**RIDASCREEN<sup>®</sup> FAST DON**

manufactured by

**R-Biopharm AG**

**An der neuen Bergstraße 17**

**64297 Darmstadt**

**Germany**

This method has been evaluated in the AOAC<sup>®</sup> *Performance Tested Methods<sup>SM</sup>* Program, and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC<sup>®</sup> Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested<sup>SM</sup>* certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (November 11, 2018 – December 31, 2019). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

*Scott Coates*

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Scott Coates, Senior Director  
Signature for AOAC Research Institute

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November 11, 2018

Date

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<b>KIT NAME(S)</b> RIDASCREEN® FAST DON	<b>CATALOG NUMBERS</b> R5901, R5902
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<b>INDEPENDENT LABORATORY</b> Trilogy Analytical Laboratory, Inc. 870 Vossbrink Dr. Washington, MO 63090 USA	<b>AOAC EXPERTS AND PEER REVIEWERS</b> Gary Lombaert <sup>1</sup> , David M. Wilson <sup>2</sup> , Mary Trucksess <sup>3</sup> <sup>1</sup> Health Protection Branch, Winnipeg, Canada <sup>2</sup> University of Georgia, Department of Plant Pathology, Tifton, GA, USA <sup>3</sup> US FDA, CFSAN, Washington, DC, USA
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<b>APPLICABILITY OF METHOD</b> Target analyte – Deoxynivalenol (DON, also cited as vomitoxin)  Matrices – wheat, barley, malted barley, oats, corn	<b>REFERENCE METHOD</b> “HPLC Reference Method for deoxynivalenol in wheat-working instruction” prepared by U.S. Department of Agriculture, Grain Inspection Packers and Stockyards Administration Technical Service Division, version of 5-6-1997 (2)
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**Performance claims - The performance characteristics of RIDASCREEN® FAST DON meet the specification as requested by the U.S. Department of Agriculture:**

- 1) Time required for completion of an analysis of a pre-ground sample is less than 30 minutes.
- 2) Limit of detection is well below 0.5 ppm.
- 3) Accuracy and precision with fortified samples (fortification levels: 0, 0.5, 1.0, 2.5, and 5.0 ppm) meet the requirements.
- 4) Accuracy measurement of naturally contaminated wheat samples in comparison with the official HPLC method result in good agreement of the values obtained by ELISA and by HPLC.
- 5) The ELISA is not sensitive to temperature changes between 18 and 30 °C.
- 6) The test kit components are stable as indicated on the test kit labels.

<b>ORIGINAL CERTIFICATION DATE</b> July 12, 2000	<b>CERTIFICATION RENEWAL RECORD</b> Renewed Annually through December 2019
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<b>METHOD MODIFICATION RECORD</b> 1. December 2017 Level 1	<b>SUMMARY OF MODIFICATION</b> 1. Editorial changes
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<b>Under this AOAC® Performance Tested<sup>SM</sup> License Number, 000701 this method is distributed by:</b> NONE	<b>Under this AOAC® Performance Tested<sup>SM</sup> License Number, 000701 this method is distributed as:</b> NONE
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**PRINCIPLE OF THE METHOD (1)**  
The basis of the test is the antigen-antibody reaction; a deoxynivalenol specific antibody developed in a rabbit is used for the detection of the analyte. The microtitration plate is coated with sheep anti-rabbit IgG antibody as catching antibody. Deoxynivalenol standard or sample, Deoxynivalenol-enzyme conjugate and rabbit anti-Deoxynivalenol antibody are added. The mixture is incubated for 5 min. Deoxynivalenol and Deoxynivalenol-enzyme conjugate compete for the antibody-Deoxynivalenol binding site (competitive enzyme immunoassay). At the same time, the rabbit antibody is bound by the immobilized sheep antibody. Any not bound enzyme conjugate is then removed by a washing step. Chromogen/substrate is added to the wells and incubated for 3 min. Bound enzyme converts the chromogen into a blue product. The addition of the stop reagent inhibits the enzymatic process and causes a shift of the colored product to yellow. Measurement is performed photometrically at 450 nm (optional reference wavelength  $\mu$  600 nm). The resulting absorbance values are inversely proportional to the concentration of Deoxynivalenol of the sample.

**DISCUSSION OF THE VALIDATION STUDY (1)**  
The RIDASCREEN® FAST DON test kit investigated was proven being highly reproducible and accurate. The test was insensitive against changes of the ambient temperature between 18 and 30 °C. Samples naturally contaminated with DON resulted in nearly identical values with the ELISA and with HPLC used as reference method.

**Table 4. Analysis of sample P102/3, Comparison of ELISA and HPLC (1)**  
**ELISA results referred are mean values (ppm) of triplicate measurements**

sub-portion no.	ELISA				HPLC
	analyst 1 conc. (ppm)	analyst 2 conc. (ppm)	analyst 3 conc. (ppm)	over all (n=15)	single results conc. (ppm)
1	0.868	0.798	0.898		0.7
2	0.778	0.675	0.823		0.7
3	1.011	1.049	1.223		
4	0.707	0.913	0.742		
5	0.730	0.682	0.715		
mean				0.841	0.7
standard deviation. (ppm)				0.156	
CV (%)				18.6	

**Table 5. Analysis of sample A2/2, Comparison of ELISA and HPLC (1)**  
**ELISA results referred are mean values (ppm) of triplicate measurements**

sub-portion no.	ELISA				HPLC
	analyst 1 conc. (ppm)	analyst 2 conc. (ppm)	analyst 3 conc. (ppm)	over all (n=15)	single results conc. (ppm)
1	3.848	4.274	4.576		3.8
2	4.020	4.585	4.419		3.9
3	4.168	4.066	3.626		
4	3.604	3.732	3.781		
5	3.772	4.065	3.760		
mean				4.020	3.85
standard deviation. (ppm)				0.328	
CV (%)				8.2	

**Table 6. Calculation of the limit of detection (LOD) and of limit of quantitation (LOQ) (1)  
Results (DON concentration, ppb) of ten measurements of each matrix**

replicate	wheat	barley	malted barley	oats	corn
1	15.205	10.498	52.137	89.062	32.062
2	22.217	1.439	53.269	92.999	23.273
3	17.563	7.938	31.068	52.891	24.039
4	22.217	0.000	20.021	52.891	4.825
5	57.955	6.483	17.212	100.631	34.477
6	38.299	1.266	24.349	42.768	32.957
7	31.331	8.186	15.255	56.341	14.988
8	27.505	0.471	10.478	69.840	30.308
9	6.961	6.483	35.126	88.577	46.487
10	23.834	3.573	48.077	134.047	32.957
conc., mean (ppb)	26.309	4.634	30.699	78.005	27.637
s. d. (ppb)	14.086	3.745	15.897	28.187	11.537
<b>LOD (ppb) mean + 2 x s.d.</b>	<b>54</b>	<b>12</b>	<b>62</b>	<b>134</b>	<b>51</b>
<b>LOQ (ppb) mean + 10 x s.d.</b>	<b>167</b>	<b>42</b>	<b>190</b>	<b>360</b>	<b>143</b>

#### REFERENCES CITED

1. Bernhard Reck, Evaluation of the RIDASCREEN® FAST DON, AOAC® *Performance Tested*<sup>SM</sup> certification number 000701.
2. "HPLC Reference Method for deoxynivalenol in wheat-working instruction" prepared by U.S. Department of Agriculture, Grain Inspection Packers and Stockyards Administration Technical Service Division, version of 5-6-1997