



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

000701

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

RIDASCREEN[®] FAST DON

manufactured by

R-Biopharm AG

An der neuen Bergstraße 17

64297 Darmstadt

Germany

This method has been evaluated in the AOAC[®] *Performance Tested Methods*SM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*SM 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 (January 07, 2021 – December 31, 2021). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

January 07, 2021

Date

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KIT NAME(S) RIDASCREEN® FAST DON	CATALOG NUMBERS R5901, R5902
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INDEPENDENT LABORATORY Trilogy Analytical Laboratory, Inc. 870 Vossbrink Dr. Washington, MO 63090 USA	AOAC EXPERTS AND PEER REVIEWERS Gary Lombaert ¹ , David M. Wilson ² , Mary Trucksess ³ ¹ Health Protection Branch, Winnipeg, Canada ² University of Georgia, Department of Plant Pathology, Tifton, GA, USA ³ US FDA, CFSAN, Washington, DC, USA
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APPLICABILITY OF METHOD Target analyte – Deoxynivalenol (DON, also cited as vomitoxin) Matrixes – wheat, barley, malted barley, oats, corn	REFERENCE METHOD “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.

ORIGINAL CERTIFICATION DATE July 12, 2000	CERTIFICATION RENEWAL RECORD Renewed Annually through December 2021
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METHOD MODIFICATION RECORD 1. December 2017 Level 1	SUMMARY OF MODIFICATION 1. Editorial changes
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Under this AOAC® Performance TestedSM License Number, 000701 this method is distributed by: NONE	Under this AOAC® Performance TestedSM License Number, 000701 this method is distributed as: 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 μ 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
LOD (ppb) mean + 2 x s.d.	54	12	62	134	51
LOQ (ppb) mean + 10 x s.d.	167	42	190	360	143

REFERENCES CITED

1. Bernhard Reck, Evaluation of the RIDASCREEN® FAST DON, AOAC® *Performance TestedSM* 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