CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

120601

The AOAC Research Institute hereby certifies the method known as:

RIDASCREEN® Gliadin

manufactured by

R-Biopharm AG
An der neuen Bergstraβe 17
64297 Darmstadt
Germany

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 3, 2022
Expiration Date	December 31, 2023

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METHOD NAME
RIDASCREEN® Gliadin

CATALOG NUMBER
R7001

INDEPENDENT LABORATORY
19 European Laboratories
1 Argentinean Laboratory

APPLICABILITY OF METHOD
Target analyte – Gliadin, Secalins, and Hordeins

Matrixes – (1 g) – wheat, buckwheat, rice, corn, oats, syrup, and sausage

Performance claims – The performance characteristics of RIDASCREEN® Gliadin meet the following specification:
1) Time required for completion of the cocktail sample preparation was 100 minutes and less than 100 minutes for the test implementation.
2) Specificity of the test with naturally Gluten-free blank samples was 100 % (no false positive samples were detected)
3) Analytical Sensitivity was found at LOD < 1.5 ppm Gliadin as measured by 10-fold determination of various Gluten-free samples. LOQ was set at 2.5 ppm Gliadin, which was confirmed by multiple measurement of a sample contaminated close to that value.

Performance claims cont.
4) Accuracy measurement
Accuracy was investigated with bread samples fortified at defined levels between 30 and 150 ppm Gliadin. Mean recovery was found at 107.8 ppm +/- 16.7 ppm.
5) The ELISA is not sensitive to temperature changes between 18 and 37 °C.
6) The ELISA was not sensitive to variation of incubation time between 3x 25 and 3x 35 minutes.
7) The ELISA was not sensitive to small changes of reagent volumes between 90 and 110 µl.
8) The test kit components are stable as indicated on the test kit labels (shelf life is usually more than 12 month).

ORIGINAL CERTIFICATION DATE
December 12, 2006

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2023.

METHOD MODIFICATION RECORD
1. **March 2017 Level 2**
2. **December 2022 Level 1**

SUMMARY OF MODIFICATION
1. **Change to a mercury-free preserving agent in the washing buffer.**
2. **Editorial changes.**

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NONE

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NONE

PRINCIPLE OF THE METHOD (1)
The basis of the test is an antigen-antibody reaction. A Gliadin specific antibody developed as mouse monoclonal R5-antibody is used for the detection of the analyte. A microtiter plate is coated with monoclonal R5-antibody. Gliadin standard or samples are incubated for 30 minutes. After washing, the antibody-(R5) enzyme conjugate is added for further 30 minutes. The conjugate binds to the Gliadin – antibody complex on the plate (sandwich enzyme immunoassay). Any not bound enzyme conjugate is then removed by a washing step. Chromogen/substrate is added to the wells and incubated for 30 min. Bound enzyme converts the chromogen into a blue colored product. The addition of 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 proportional to the concentration of Gliadin of a sample.

DISCUSSION OF THE VALIDATION STUDY (1)

The RIDASCREEN® Gliadin test kit investigated was proven being highly reproducible and accurate with respect to samples fortified between 24 and 139 ppm Gliadin in an internal in-house study and in an international ring trial.

No false negative or false positive samples were found during these studies.

The stability of the test was demonstrated on the example of two batches over observation periods of 49 and 67 months. The test did not show any loss of its analytical capacity.

LOD values measured on the basis of 6 x 10 blank samples of various matrixes were calculated well below standard 2. Mean value + 3 x standard deviation of the 6 sets of blank samples was found below 1.5 ppm Gliadin.

The LOQ of 2.5 ppm was investigated with a contaminated sample close to 2.5 ppm Gliadin and found at a mean value of 2.46 +/- 0.195 (CV = 7.9%). That result confirmed the defined LOQ and proved that samples measured at the LOQ differed significantly from zero.

An in house lot-to-lot study on the basis of 4 batches of the test kit performed with control samples and bread samples fortified on defined levels of Gliadin resulted in a mean recovery value of 107.6 %.

The test was insensitive against changes of the ambient temperature between 18 and 37 °C; against variation of incubation time +/-5 minutes to the regular incubation time of 30 minutes and against variation of volume pipetted in each incubation step (100µl +/- 10 µl).

Cross reactivity was tested with a variety of 40 compounds including grains, vegetables, and nuts. All samples were found close to standard 1; no cross reactivity was observed.

An independent evaluation study was performed under the leadership of the Working Group on Prolamin Analysis and Toxicity (WGPAT) on the basis of 12 encoded samples consisting of 7 spiked samples (four heat treated and three not heat treated samples) and 5 commercially purchased samples. Four of the samples were naturally Gluten-free samples.

All spiked samples have been prepared in the University of Munich, whereas 5 samples (no. 8 – 12) were provided by Centro National de Biotecnologia, Universidad Autonoma Cantoblanco, Madrid, Spain. The maize dough samples have been spiked with the PWG reference standard and were baked in a research bakery. All samples were dried and milled to a homogeneous powder, which was provided for the ring trial.

Rice flour was fortified on two defined levels (high and low level) and the homogenized samples were provided to the ring trial as well.

The negative samples were all found below standard 2 (< 2.5 ppm), except sample 4, which obviously was contaminated during the bakery process at a low level of Gliadin (mean 8.7 ppm). The sample was excluded from statistical calculation.

Mean recovery value (97.4%) of the spiked samples was excellent and found in the same range like the in house study showed (107.6 %). The repeatability (18%) and reproducibility (30 %) was found in the usual range of ELISA tests.

Table 8. Limit of detection with raspberry syrup (1)
Analysis of ten blank samples/ run

Sample ID	*Matrix*	*Absorbance*	Gliadin (ppm)*
G-333-1	*Rasberry syrup*	0.061	0.0
G-333-2		0.062	0.0
G-333-3		0.062	0.0
G-333-4		0.064	1.0
G-333-5		0.064	1.0
G-333-6		0.073	1.1
G-333-7		0.062	0.0
G-333-8		0.060	0.0
G-333-9		0.054	0.0
G-333-10		0.065	1.0
Mean		**0.063**	**1.0**
SD		**0.0047**	**0.058**
3x SD		**0.014**	**0.174**
LOD (mean + 3SD)		**1.17 ppm**	

*extrapolated values

Table 9. Limit of detection with a pure kind of oats (1)
Analysis of ten blank samples/ run

Sample ID	*Matrix*	*Absorbance*	Gliadin (ppm)*
C-2-1	*Pure variety of oats (Lutz)*	0.093	1.2
C-2-2		0.083	1.1
C-2-3		0.087	1.1
C-2-4		0.079	1.1
C-2-5		0.100	1.2
C-2-6		0.089	1.1
C-2-7		0.100	1.2
C-2-8		0.091	1.2
C-2-9		0.106	1.2
C-2-10		0.100	1.2
Mean		**0.093**	**1.2**
SD		**0.01**	**0.0516**
3x SD		**0.03**	**0.1548**
LOD (mean + 3SD)		**1.36 ppm**	

*extrapolated values

Table 10. Limit of detection with buckwheat flour (1)
Analysis of ten blank samples/ run

Sample ID	*Matrix*	*Absorbance*	Gliadin (ppm)*
G-500-1	*Buckwheat flour*	0.108	0.9
G-500-2		0.119	0.96
G-500-3		0.098	0.84
G-500-4		0.119	0.96
G-500-5		0.102	0.86
G-500-6		0.130	1.03
G-500-7		0.103	0.87
G-500-8		0.119	0.96
G-500-9		0.095	0.82
G-500-10		0.113	0.92
Mean		**0.111**	**0.912**
SD		**0.0112**	**0.1379**
3x SD		**0.034**	**0.414**
LOD (mean + 3SD)		**1.33 ppm**	

*extrapolated values

Table 11. Limit of detection with Basmati rice (1)
Analysis of ten blank samples/ run

Sample ID	*Matrix*	*Absorbance*	Gliadin (ppm)*
G-198-1	*Basmati rice*	0.098	1.0
G-198-2		0.067	1.0.
G-198-3		0.067	1.0
G-198-4		0.064	1.0
G-198-5		0.071	1.1
G-198-6		0.072	1.1
G-198-7		0.074	1.1
G-198-8		0.064	1.0
G-198-9		0.068	1.0
G-198-10		0.064	1.0
Mean		**0.068**	**1.0**
SD		**0.0035**	**0.048**
3x SD		**0.011**	**0.144**
CV (%)			**4.8**
LOD (mean + 3SD)		**1.14 ppm**	

*extrapolated values

able 12. Limit of detection with a defined sausage sample
(produced at a butcher under defined conditions) (1)
Analysis of ten blank samples/ run

Sample ID	*Matrix*	*Absorbance*	Gliadin (ppm)*
G-208-1	*sausage*	0.074	1.1
G-208-2		0.089	1.1
G-208-3		0.072	1.1
G-208-4		0.094	1.2
G-208-5		0.068	1.0
G-208-6		0.075	1.1
G-208-7		0.067	1.0
G-208-8		0.071	1.1
G-208-9		0.066	1.0
G-208-10		0.068	1.0
Mean		**0.074**	**1.1**
SD		**0.01**	**0.067**
3x SD		**0.03**	**0.201**
LOD (mean + 3SD)		**1.30 ppm**	

*extrapolated values

Table 13. Limit of detection with corn (1)
Analysis of ten blank samples/ run

Sample ID	*Matrix*	*Absorbance*	Gliadin (ppm)*
G-608-1	*corn*	0.337	1.13
G-608-2		0.319	0.89
G-608-3		0.296	0.65
G-608-4		0.323	0.94
G-608-5		0.316	0.85
G-608-6		0.309	0.77
G-608-7		0.305	0.73
G-608-8		0.346	1.27
G-608-9		0.330	1.03
G-608-10		0.331	1.04
Mean		**0.321**	**0.930**
SD		**0.015**	**0.191**
3x SD		**0.045**	**0.574**
LOD (mean + 3SD)		**1.5 ppm**	

*extrapolated values

REFERENCE CITED

1. Immer, Ulrike, & Bernhard Reck, Evaluation of the RIDASCREEN® Gliadin, AOAC *Performance Tested Methods*^{SM} certification number 120601.
2. R-Biopharm, Evaluation of Requested exchange of thimerosal in RIDASCREEN® Gliadin R7001, Approved March 2017.