R-Biopharm AG

RIDASCREEN® Gliadin

Art.No. R7001

- AOAC-Official Method of Analysis (2012.01) New
- AOAC-RI certified (120601)
- Codex Alimentarius Method (Type I)

Validation Report

12/2012

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Test validation

RIDASCREEN® Gliadin is a sandwich enzyme immunoassay for the quantitative analysis of prolamins from wheat (gliadin), rye (secalin) and barley (hordein). It should be used for contamination control of dietary products for celiac patients.

RIDASCREEN® Gliadin (R7001) is approved by the AOAC as Official First Action Method. The method was assigned AOAC Official Method number 2012.01. The test kit is also a Performance Tested Method of the AOAC Research Institute (AOAC-RI 120601).
The R5 ELISA has been collaboratively tested and was endorsed as type I method by the CODEX Alimentarius.

Calibration curve

The quantification of the prolamins relies on comparison of test antigen responses with those of a series of standard dilutions.

The RIDASCREEN® Gliadin test kit is calibrated to the WGPAT gliadin material (PWG gliadin), a highly purified gliadin preparation (86 %) from 40 different European wheat varieties. This reference material was produced on behalf of the European Working Group on Prolamin Analysis and Toxicity (WGPAT). It was isolated from the 10 most frequently grown wheat varieties from France, Germany and Britain.

A typical standard curve for RIDASCREEN® Gliadin (R7001) is shown in appendix 1. The calculation of the gluten content is based on the assumption of a 1:1 ratio between gliadin and glutenin. The result is read from the curve as ng/ml prolamin and has to be multiplied by the dilution factor of the sample. If the result should be expressed as gluten, the result has to be multiplied by 2.

Specificity of the R5 antibody

The monoclonal antibody R5 reacts with all gliadin-fractions from wheat and with the prolamins from rye and barley. The R5 reacts also to wheat-like cereals like spelt or kamut.

No cross-reactivities have been observed to soy, oats, corn, rice, millet, buckwheat, quinoa and amaranth.

Further detailed information can be found in the separate document **"Specificity of the R5 antibody employed in the Gliadin product line"**.

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Sensitivity

The **Limit of Detection** or the lowest detectable level that can be distinguished from zero matrices was determined at 1.2 mg/kg for the RIDASCREEN® Gliadin (see table 1). This value is close to 1.5 mg gliadin/kg sample, which is indicated in the test kit insert.
The 99 % confidence level for the RIDASCREEN® Gliadin is 1.19 +/- 0.22 mg/kg (3 times the standard deviation).

Table 1 Determination of the limit of detection of RIDASCREEN® Gliadin by measuring each matrix ten fold

Sample	Gliadin Concentration (mg/kg) (Mean of a 10 fold determination)	3 fold Standard Deviation	LOD (mean + 3 SD)
Raspberry syrup	1.00	0.174	**1.17**
Maize	1.53	0.093	**1.62**
Pure kind of oats	1.20	0.155	**1.35**
Buckwheat flour	0.91	0.414	**1.33**
Sausage	1.10	0.201	**1.30**
Corn	0.93	0.574	**1.50**
Golden Sun Rice	1.52	0.121	**1.64**
Thai Rice	1.48	0.114	**1.59**
Basmati Rice	1.00	0.144	**1.14**
Mean	**1.19**	**0.221**	**1.40**

The **Limit of Quantification** or the lowest concentration that can be determined in a sample with acceptable precision (repeatability) and accuracy under the stated conditions of the test was determined at 2.5 mg/kg gliadin corresponding 5 mg/kg gluten.

Precision (scatter of replicate readings around their mean value)
To determine the reproducibility and repeatability of the RIDASCREEN® Gliadin the inter- and intra-assay coefficients were calculated.

Intra-Assay Variation (repeatability)
Within run variation was calculated by determination of the standards of one batch. The coefficient of variation within one batch varied between 3.2 % and 5.2 % (see table 2 a).

Table 2a Intra-Assay Variation within one batch at different dates (n=8)

RIDASCREEN® Gliadin batch 03426	Coefficient of Variation in %
2006-12-22	3.9
2007-01-09	4.2
2007-04-09	3.2
2007-07-16	3.9
2007-11-01	5.2
2008-01-10	4.6
2008-04-10	3.3

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The Intra-Assay Variation was not only determined for the standards but also for samples as can be seen in table 2b.

Table 2b Intra-Assay Variation for RIDASCREEN® Gliadin (n = 6 replicates)

Sample Description	Sample Concentration (mg/kg gliadin)	Standard Deviation	Coefficient of Variation (in %)
Maize flour (contaminated)	1.2	0.03	2.5
Bread maize (spiked)*	24	0.69	2.9
Bread maize (spiked)*	56	1.5	2.7

* The dough of the bread was spiked with WG PAT Gliadin. Thereafter, the bread was baked.

Inter-Assay Variation (reproducibility)
Between run variation was determined by replicate measurements of the standards from different test kits of the same production batch. The coefficient of variation CV (%) from batch to batch expressed as a mean of the 6 standard solutions for the different production batches is between 4.5 and 6.5 % as can be seen in the table 3. These low CV are an indicator for an excellent batch to batch production stability resulting in a high quality of test results.

Table 3 Comparison of mean coefficient of variation (%) for the standards during the whole shelf-life from batch to batch

RIDASCREEN® Gliadin batch	Coefficient of Variation in % (n = 6)
02499	4.8
01499	6.5
03109	5.5
01258	4.7
03477	5.0
02067 (A)	4.5
03426	3.8

The Cocktail (patented) (R7006) is recommended for the sample preparation. This extraction procedure was developed by Méndez et al (Patent PCT WO 02/09 2633A1).

Sample preparation

The Cocktail (patented) (R7006) is recommended for sample preparation. This extraction procedure was developed by Mendez et.al. (Patent PCT WO 02/09 2633A1).

Detailed information can be found in the separate document
„Extraction efficiencies for gliadin analysis“.

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Recoveries of spiked samples

To measure the accuracy a bread dough was spiked at different gliadin levels with PWG gliadin (WGPAT). After baking, the bread was ground dry, extracted with Cocktail (patented) (R7006) and measured with the RIDASCREEN® Gliadin. The recovery lies between 100 and 113 % (table 4).

Table 4 Bread samples spiked with PWG gliadin before baking and extracted with Cocktail (patented)

Sample	Gliadin Spike mg/kg (ppm)	Gliadin measured mg/kg (ppm)	Recovery (%)
Bread-1	139	153.4	110
Bread-2	58	60.2	104
Bread-5	77	86.7	113
Bread-6	24	26.5	110
Bread-7	33	32.8	99.9

Various matrices have been spiked with different PWG gliadin concentrations. Table 5 shows excellent recoveries for different types of heated and unheated samples that were spiked with PWG gliadin and extracted with Cocktail (patented).

Table 5 Recoveries of different processed samples extracted with Cocktail (patented)

Sample	Status	Gliadin spike mg/kg (ppm)	Gliadin measured mg/kg (ppm)	Recovery (%)
Sausage A	unheated	0	< 2.5	
Sausage A	unheated	20	19.9	100
Sausage A	heated	20	18.6	93
Rice flour I	unheated	0	< 2.5	
Rice flour I	unheated	10	10.0	100
Rice flour I	unheated	20	18.3	92
Sausage B	unheated	20	22.5	113
Sausage B	heated	10	10.7	107
Sausage B	heated	20	21.4	107
Maize flour A	unheated	10	9.6	96
Maize flour A	unheated	20	18.7	94
Maize flour A	heated	10	10.9	109
Maize flour A	heated	20	17.7	96
Sausage C	unheated	10	9.6	96
Sausage C	unheated	20	20.0	100
Sausage C	heated	10	9.0	90
Sausage C	heated	20	19.5	98
Rice flour II	unheated	10	9.5	95
Rice flour II	unheated	20	20.1	101
Rice flour II	heated	20	18.6	93

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Results of the collaborative study organized by WGPAT

RIDASCREEN® Gliadin (R7001) was successfully tested in an international collaborative study organized by the Working Group on Prolamin Analysis and Toxicity (WGPAT). Twenty laboratories participated in the study. Part of the samples were spiked with PWG gliadin before baking and extracted with Cocktail (patented). The results of the raw materials and processed food samples with concentrations between 0 and 200 ppm are shown in table 6. Rice is naturally gluten-free and gives consequently results below the detection limit otherwise the rice sample is contaminated. The maize flour (sample 4) has been tested negative before making the dough. During processing a contamination of the sample might have occurred when adding the yeast. A low gliadin contamination for sample 4 was measured (this result was confirmed from a second test from a further kit supplier).

The results of the collaborative study are also published (Mendez E. , Vela C., Immer U. and Janssen F.W. (2005): Report of a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food: European Journal of Gastroenterology & Hepatology 2005, 17:1053-1063).

Table 6 R-Biopharm results: Recoveries of the collaborative study for RIDASCREEN® Gliadin (R7001)

ID	Sample	Status	PWG gliadin spiked mg/kg (ppm)	Gliadin measured mg/kg (ppm)	Recovery (%)
PWG-1	maize	heated	168	134	**80**
PWG-2	maize	heated	35	33	94
PWG-3	maize	heated	79	71	90
PWG-4	maize*	heated	(0)*	8.7	
PWG-5	rice	non heated	41	36	88
PWG-6	rice	non heated	0	-	
PWG-7	rice	non heated	147	112	76
PWG-8	cont. wheat starch	non heated	14	14	100
PWG-9	cont. rice flour	non heated	13	16	123
PWG-10	cont. wheat starch	non heated	(12-15)	15	111
PWG-11	cont. maize flour	on heated	<1.5	-	
PWG-12	cont. maize flour	non heated	<1.5	-	

*contaminated during bakery process

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Stability of the test

The stability of the test is routinely checked by R-Biopharm's quality assurance laboratory after defined storage intervals. Test kits are stored in a cold room at temperatures of 2 - 8 °C (35 - 46 °F). Before testing the kit components are temperature equilibrated to room temperature (20 - 25 °C / 68 77 °F). The absorbance values of standards and control samples were found stable during the observation time of 18 months. Real time stability of the test will regularly be controlled according to the total quality management schedule of the company.

Interferences and Sources of Error

The RIDASCREEN® Gliadin product line is very sensitive. Before carrying out an ELISA the lab hygiene should be checked with a RIDA®QUICK Gliadin (R7003). If the laboratory is contaminated with cereal dust it should be cleaned with ethanol (60 %) or propanol. It is of prime importance that the sample preparation for the ELISA is carried out in a separate room from the ELISA procedure.

Often customers obtain positive results for oat, maize or buckwheat. The reason can be that other cereals lead to a cross contamination by dust. Barley is in most cases the main contaminant in oat based products due to similar harvesting times. Contamination can be detected with the R5 antibody in each case.

Conclusion

In conclusion, the R5-ELISA is viewed as the basis for a new generation of robust ELISA kits with excellent sensitivity, specificity and accuracy. The system is equally sensitive to barley, wheat and rye prolamins. The high sensitivity of R5-ELISA allows the determination of gluten levels in foods down to 3 mg/kg gluten, which is well below the 20 mg/kg threshold of the Codex Alimentarius Commission. For quantitative gluten measurements the Cocktail (patented) (R7006) or RIDA® Extraction Solution (R7099) should be used.

The RIDASCREEN®Gliadin ELISA is a simple and sensitive detection method which can be used for fast routine testing. The R5 method is accepted by the Codex Alimentarius as Type I Method and has been successful approved by the AOAC Research Institute as performance tested method (Certf. No. 120601).

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List of references

1. Seilmeier W., Wieser H. (2003) Comparative investigations of gluten proteins from different wheat species: Eur Food Res Technol 217:360-364
2. Valdés I., Garcia E., Llorente M., Mendez E. et al. (2003) Innovative approach to low-level gluten determination in foods using a novel sandwich enzyme-linked immunosorbent assay protocol: Eur. Journal of Gastroenterology & Hepatology Vol 15 No 5
3. Mendez E. , Vela C., Immer U. and Janssen F.W. (2005) Report of a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food: European Journal of Gastroenterology & Hepatology 2005, Vol. 17 No 10

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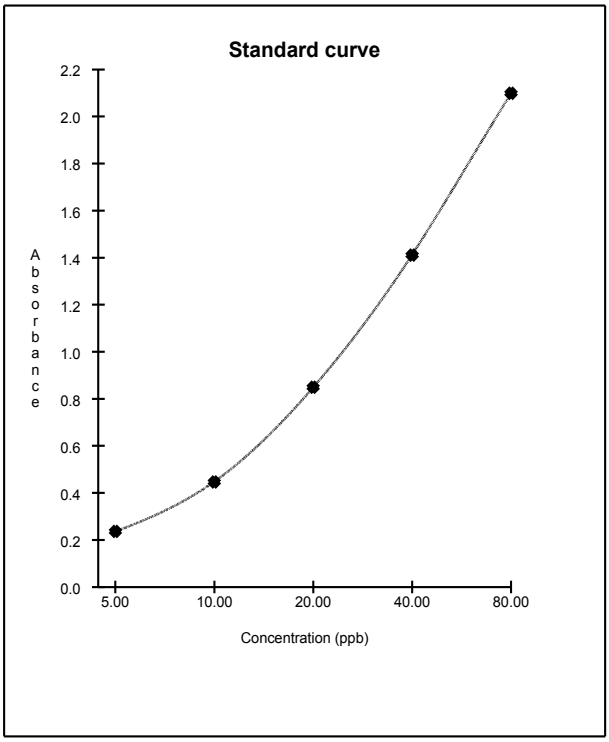
QUALITY ASSURANCE CERTIFICATE

RIDASCREEN® Gliadin

Art. No.: R7001 Lot: 02499 Expiry: 2011-03

R-Biopharm AG, Darmstadt, Germany certifies that this batch has been approved by the Quality Assurance Department and conforms with specifications



Standards

Std. Conc.(ppb) CV(%)	n mean
Std1 0.00 11.5	8 0.048
Std2 5.00 3.9	8 0.237
Std3 10.00 4.2	8 0.447
Std4 20.00 4.3	8 0.849
Std5 40.00 2.6	8 1.411
Std6 80.00 3.1	8 2.099

	Lot No.	Expiry
Microwell plate	03369	2011-05
Standards	13120	2011-11
Conjugate	02479	2011-07
Buffer1	03459	2011-07
Substrate/Chromogen	03408	2011-03
Stop solution	04349	2014-07
Washing buffer	04419	2012-03

Please note:

The absorbance for the standards may decrease during the shelf life of the kit. The general shape of the curve will remain similar, while the slope might change slightly. Furthermore refer to product leaflet 8. Indication of instability or deterioration of reagents.

sign.: Edda Rohm
Quality Assurance Representative

Date: 2010-03-25

Remark:
This document has been created electronically and is therefore valid without a signature.

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