RIDA®QUICK Gliadin

Art. No. R7003/R7004/R7005

Product information

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General

RIDA®QUICK Gliadin uses the R5 antibody for gliadin detection also used in the ELISAs RIDASCREEN® Gliadin (Art. No. R7001), RIDASCREEN®FAST Gliadin (Art. No. R7002), RIDASCREEN®FAST Gliadin sensitive (Art. No. R7051) and RIDASCREEN® Gliadin competitive (Art. No. R7021). The official type I method for gluten determination according to the Codex Alimentarius is an ELISA which uses the R5 antibody (Mendez). This requirement is fulfilled by the sandwich ELISA RIDASCREEN® Gliadin (R7001). The test strips of RIDA®QUICK Gliadin show a good correlation with the official method, the R5-ELISA RIDASCREEN® Gliadin. R-Biopharm AG is the only company that is allowed to market a R5 dip stick.

The monoclonal R5-antibody detects specifically gliadin from wheat and prolamins from rye and barley by formation of a sandwich structure (antibody – antigen – antibody). The dip stick can be used directly for swabbing and analysis of cleansing water (e.g. clean-in-place water). Additionally, also food samples can be analysed after extraction of gluten from samples.
Generally, the higher the analyte level in the sample, the stronger the red color of the test band will be. The test has been developed for the detection of low amounts of gluten (contamination). No high-dose-hook-effect is observed at high concentrations. However, the red target band may smear at high gluten concentrations.

The dip stick RIDA®QUICK Gliadin (R7003) was approved by the AOAC Performance Tested Methods℠ Program and was assigned **PTM Certification No. 101702** by the AOAC Research Institute for analysis of surfaces and cleansing waters.
The in-house validation data were confirmed by an independent lab and demonstrated that the dip stick is applicable for the detection of traces of gliadin on surfaces (stainless steel, sealed ceramic, plastic and silicone rubber) and in clean-in-place waters.

The RIDA®QUICK Gliadin test kit was also evaluated in an international collaborative study for analysis of food samples. The assay is an AACC international approved method (**38-60.01**) and an AOAC approved Official Method of Analysis (**Final Action OMA 2015.16**) for the analysis of food samples.

Weblinks

http://www.aoac.org/aoac_prod_imis/AOAC_Docs/RI/18PTM/18C_101702_rbiogliadin.2.pdf
http://www.eoma.aoac.org/methods/info.asp?ID=51131
http://methods.aaccnet.org/summaries/38-60-01.aspx

The Limit of Detection was evaluated for the following matrices:

- Surfaces approx. 1.6 - 3 µg gluten/100 cm^2
- Raw material approx. 4.4 mg/kg gluten
- Processed food approx. 6.3 mg/kg gluten
- Cleansing water (without cleansing reagent) approx. 10 ng/ml gluten
- Cleansing water (with cleansing reagent) approx. 50 - 100 ng/ml gluten

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RIDA®QUICK Gliadin is available in 3 different formats (the dip sticks are identical only the packaging and accessories differ):

- **R7003 RIDA®QUICK Gliadin** – contains 25 strips in a reclosable tube, 25 plastic pipettes, 30 vials and buffer
- **R7004 RIDA®QUICK Gliadin (single packaged)** – contains 25 strips single packaged, 30 vials and buffer
- **R7005 RIDA®QUICK Gliadin (ready to swab)** – contains 25 strips single packaged and 25 vials filled with buffer

If not stated otherwise, all concentrations in this report are in gluten (factor 2 was used to calculate from gliadin to gluten content if necessary).

Sample preparation

Surface swab

RIDA®QUICK Gliadin (Art. No. R7003/R7004/R7005) can be directly used as a swab test for surfaces without any sample preparation (swab, dip, read). For the analysis of food samples, an extraction is necessary.

One of the key points in gluten analysis is the extraction procedure. In their native form, the prolamines which are primarily detected by the R5 antibody, are monomeric proteins and contain only intramolecular disulfide bonds. In this form, the proteins can be easily extracted with 60 % ethanol. Upon heating, the disulfide bonds get re-arranged and the prolamines form big aggregates with other gluten proteins. These aggregates can only be extracted with reducing agents and in the presence of disaggregating agents. The Cocktail (patented) (R7006/R7016) contains beta-mercaptoethanol as reducing agent and also a disaggregating agent. It is therefore suitable for the extraction of raw and heated material. Extraction with Cocktail (patented) is the official extraction method according to Codex Alimentarius and AOAC. It is recommended for analysis with regard to compliance with legal thresholds.

As a fast and environmental-friendly screening method, RIDA® Cocktail ECO (R7080) was developed. No chemical hood is necessary for sample extraction. RIDA® Cocktail ECO contains an environmental-friendly reducing agent and disaggregating agents. Extraction of 10 samples takes approx. 35 min compared to 2 hours for Cocktail (patented). Even faster is the extraction with 60 % ethanol. However, for the reasons stated above, this extraction method should only be used for non-heated material, since it lacks reducing and disaggregating agents necessary for complete extraction of heated material.

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For tannin- and polyphenol containing samples like chocolate, coffee, cacao, buckwheat and chestnut flour, 60 % ethanol with fish gelatin should be used for extraction, (see Application note).

Often customers obtain positive results for oat, corn (maize) or buckwheat. The reason can be 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.

Another challenge are samples in which gliadin is not evenly distributed and that are difficult to homogenize e.g. oat samples. Therefore for such samples: homogenize 200 g, then carry out the extraction with at least the fourfold amount of reagents: weigh 1 g of the homogenized sample and add 10 ml of the Cocktail (patented).

To validate results spiking experiments (see appendix 1) should be carried out.

Calibration curve

The RIDA®QUICK Gliadin is a qualitative test for gliadin contamination and does not contain calibrators. Results are read visually. Generally, the higher the analyte level in the sample, the stronger the red colour of the test band will be.

Specificity of the R5 antibody

The monoclonal antibody R5 reacts with gliadin from wheat and with the prolamins from rye and barley. Wheat-like cereals (*Triticum* species) such as spelt or kamut can also be detected by the R5 antibody.

No cross-reactivities have been observed to soy, oat, corn, rice, millet, buckwheat, lupin, quinoa and amaranth. More details can be found in the document on “R 5 Gluten product line – specificity, cross reactivity and extraction efficiency”.

Sensitivity – food samples

In 2014, an international collaborative study has been conducted using the RIDA®QUICK Gliadin R7003 (*Scherf et al.*, Validation of a qualitative R5 dip stick for gluten detection with a new mathematical-statistical approach. Quality Assurance and Safety of Crops & Food, 2016; 8 (2): 309 - 318)

Two different types of samples have been used:

- Unprocessed naturally contaminated corn flour was extracted with 60 % ethanol
- Highly processed, incurred snack sample was extracted with Cocktail (patented).

18 labs participated in this collaborative study; each lab analyzed four concentrations of corn flour and four concentrations of cookie/snack samples. Each sample was analyzed in ten replicates, leading to 80 blind-coded samples per laboratory and 1440 samples in total. From these results the sensitivity was calculated.

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Table 1: Results submitted for ethanol extracted samples

Ethanol	Corn flour		Corn flour		Corn flour		Corn flour	
mg /kg gluten*	1.8		4.8		11.0		18.8	
	positive	total	positive	total	positive	total	positive	total
Grand total (18 Labs)	2	180	177	180	178	180	180	180

Table 2: Results submitted for Cocktail (patented) extracted samples

Cocktail	Cookie		Snack		Snack		Snack	
mg /kg gluten *	0.4		6.4		13.4		47.1	
	positive	total	positive	total	positive	total	positive	total
Grand total (17 Labs)	2	170	134	170	170	170	170	170

* Concentrations determined by RIDASCREEN® Gliadin R7001. Concentrations below 5 mg/kg gluten were extrapolated.

For the Cocktail (patented) extraction, one laboratory had to be excluded due to mixing up the samples.

The first corn flour sample contained a very small concentration of 1.76 mg/kg gluten which is below the limit of detection of the RIDA®QUICK Gliadin using ethanol extraction; only 2 positive results were submitted. The corn flour sample with 4.84 mg/kg gluten was already detected positive in 98.3 % of replicates.

The cookie sample with 0.38 mg/kg gluten was also clearly below the limit of detection using Cocktail (patented) extraction; only 2 positive results were submitted. The snack sample with 6.40 mg/kg gluten was already detected positive in most cases (78.8 % of replicates). The snack sample with 13.35 mg/kg gluten was clearly detected positive with 100 % positive results.

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Bubble plot for Cocktail (patented) extracted samples

The bubble plot (Figure 1) visualizes the results for the Cocktail (patented) extraction from a lab perspective. The probability of detection (percentage of positively tested samples) is plotted against the gluten concentration. The sample with a concentration of 47 mg/kg gluten was detected positive by all participating laboratories. The bubble is very large because all 17 labs reported positive results. In the case of the 6.4 mg/kg sample (which is near the cut-off) 9 labs reported only positive results, 3 labs reported 9 out 10 positive, 1 lab reported 8 out of 10 positive, another lab 7 out of 10 positive but also 2 laboratories detected only 1 out of 10 positive and 1 lab reported only negative results.

This shows that the results are highly reproducible within one lab. Differences are due to the lab-specific (individual) visual interpretation of the dip stick.

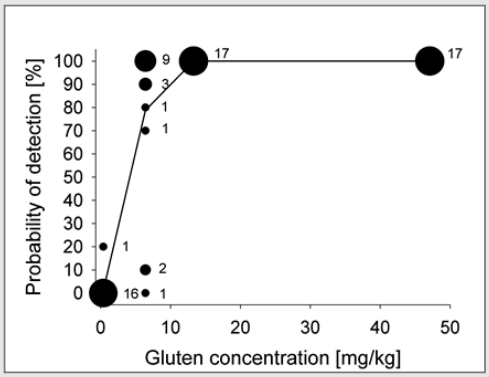

Figure 1: Bubble plot for the results obtained with Cocktail (patented) extraction (from *Scherf et al.*, Quality Assurance and Safety of Crops & Foods, 2016; 8 (2): 309 - 318)

From the data submitted it was possible to statistically calculate the Limit of Detection. For ethanol extracted sample, the minimal concentration is 4.4 mg/kg gluten with a prediction range (95 % prediction interval) of 3.5 to 5.6 mg/kg gluten.

For Cocktail (patented) extracted sample, the minimal concentration is 6.3 mg/kg gluten with a prediction range (95 % prediction interval) of 3.9 - 10.2 mg/kg gluten. The limit of detection and the variance for the extraction with Cocktail (patented) is higher due to the higher sample dilution.

Since the results of the collaborative study are very robust and reliable the dip stick is now an AACC international approved method (38-60.01) and an AOAC approved Official Method of Analysis (Final Action OMA 2015.16).

The dip stick is a qualitative method: the result is a yes or no answer. If precise quantitative results are needed, then an ELISA like RIDASCREEN® Gliadin is required.

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The results of the collaborative study matched very well with additional in-house data.

Naturally contaminated/incurred samples as well as spiked samples were used to assess the recovery and matrix suitability of the RIDA®QUICK Gliadin.

A contaminated corn flour sample (contamination probably due to cereal dust) was diluted with an uncontaminated corn flour to obtain the target values. Similarly, an incurred snack sample (contaminated with gluten prior to processing) was mixed with a comparable blank sample. Each sample was tested in 20 replicates. Gluten concentrations were determined using the RIDASCREEN® Gliadin ELISA. All samples were found positive in concentrations of 2.0 mg/kg gluten for ethanol extraction and 4.4 mg/kg gluten for Cocktail (patented) extraction.

Table 3: Naturally contaminated and incurred samples analyzed with RIDA®QUICK Gliadin

Sample matrix	Extraction	Gluten concentration [mg/kg]	Percentage of positive results
Corn flour	60 % Ethanol	0.2*	0 %
Corn flour	60 % Ethanol	2.0*	100 %
Corn flour	60 % Ethanol	4.1*	100 %
Corn flour	60 % Ethanol	9.3	100 %
Corn snack	Cocktail (patented)	0.0*	0 %
Corn snack	Cocktail (patented)	2.8*	15 %
Corn snack	Cocktail (patented)	4.4*	100 %
Corn snack	Cocktail (patented)	11.4	100 %

* Concentrations were extrapolated from the standard curve of the RIDASCREEN® Gliadin

Rice flour, soy flour, cookies and sausages were spiked at different concentrations with WGPAT gliadin (Working Group on Prolamin Analysis and Toxicity). It consists of highly purified gliadin from 40 different European wheat varieties). Rice flour and soy flour were extracted with 60 % ethanol, cookies and sausages were extracted with Cocktail (patented). Each sample was tested in 20 replicates. All samples were found positive in concentrations of 4 mg/kg gluten for ethanol extraction and 8 mg/kg gluten for Cocktail (patented) extraction.

Table 4: Different matrices spiked with the standard material from the WGPAT and analyzed by RIDA®QUICK Gliadin (results are given in % of positive results)

Gluten concentration [mg/kg]	Rice flour (ethanol extraction)	Soy flour (ethanol extraction)	Cookies (Cocktail patented extraction)	Sausages (Cocktail patented extraction)
0	0	0	0	0
1	0	0	Not tested	Not tested
2	45	100	0	0
4	100	100	0	0
8	100	100	100	100
16	100	100	100	100

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Sensitivity – surfaces and CIP water (cleansing water)

Due to the difficulty to assess a swabbing method in a collaborative study, the RIDA®QUICK Gliadin test strip was validated in an in-house study. Part of the study was repeated by an independent lab confirming the in-house results.
One important point in validating a qualitative method is to blind-code the contaminated surfaces and CIP waters before measurement. This was guaranteed by splitting the analysis in two parts.
(A) One technician contaminated the surfaces with WGPAT material or spiked the CIP waters with gluten isolate (G5004, Sigma-Aldrich). Then all surfaces or CIP waters were blindcoded.
(B) Another technician performed the dip-stick analysis and documented the read-out of the results in a blinded form prepared by the first technician.

Surfaces

Four different surfaces (10 x 10 cm) were contaminated with PWG gliadin material. After drying, the areas were analyzed by a direct swabbing of the surface with the dip-stick. After incubation of the dip-stick in buffer for 5 min, results were read visually. The outcome for 20 replicates per amount gliadin on the surface is depicted in Table 5.

Table 5: Results for four different surfaces (stainless stell, ceramic tiles, plastic, and silicone rubber) with 20 replicates for each amount of gluten that was used to contaminate each surface.

Surface	Amount µg/100 cm²	Positive n	POD* n=20
Steel	0.0	0	0.00
	0.5	5	0.25
	1.0	5	0.25
	2.0	18	0.90
	4.0	20	1.00
	8.0	20	1.00
Ceramic	0.0	1	0.05
	0.5	7	0.35
	1.0	13	0.65
	2.0	18	0.90
	4.0	20	1.00
	8.0	20	1.00
Plastic	0.0	1	0.05
	0.5	10	0.50
	1.0	17	0.85
	2.0	19	0.95
	4.0	20	1.00
	8.0	20	1.00
Silicone	0.0	0	0.00
	0.5	5	0.25
	1.0	16	0.80
	2.0	20	1.00
	4.0	20	1.00
	8.0	20	1.00

*POD = Probability of detection

Stainless-steel, plastic, sealed ceramic and silicone rubber surfaces were contaminated with gliadin; all results were positive at 4.0 µg/100 cm², 4.0 µg/100 cm², 4.0 µg/100 cm², 2.0 µg/100 cm² gluten, respectively.

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CIP water (cleansing water)

A commercial gluten preparation was used for spiking CIP waters containing three different cleansing reagents. The CIP waters were diluted 1 + 10 with sample diluent and analyzed with the dip-stick. 100 % positive results were obtained for minimum gluten concentration between 50 and 100 ng/ml (Table 6). If the CIP water does not contain cleansing reagents, the samples can be diluted 1 + 1 with sample diluent and the minimum detectable gluten level is at 10 ng/mL.

Table 6: Results for three different CIP waters that contain the surfactants Mikro Quat Classic, Acifoam or Divosan and pure water with 20 replicates for each concentration of gluten that was used to contaminate each solution.

Surfacant	Concentration ng/ml gluten	Positive n	POD* (n=20)
Mikro quad classic	0	0	0.00
	25	14	0.70
	50	18	0.90
	100	20	1.00
	200	20	1.00
Acifoam	0	0	0.00
	25	5	0.25
	50	20	1.00
	100	20	1.00
	200	18	0.90
Divosan	0	0	0.00
	25	13	0.65
	50	20	1.00
	100	20	1.00
	200	20	1.00
Water	0	0	0.00
	4.5	10	0.50
	9.1	20	1.00
	18.2	20	1.00
	36.4	20	1.00

*POD = Probability of detection

Cross reactivity

No cross-reactivities have been observed to e.g. soy, oat, corn, rice, millet, buckwheat, lupin, quinoa and amaranth. More details on cross reactivities can be found in the document on “R 5 Gluten product line – specificity, cross reactivity and extraction efficiency”.

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Samples from the market

Table 7 shows the gluten concentration of different food samples from the market extracted with 60 % ethanol. The gluten concentration was determined by ELISA using RIDASCREEN® Gliadin, RIDASCREEN®FAST Gliadin and by RIDA®QUICK Gliadin.

All test systems employ the R5-antibody which is able to quantify prolamins from wheat, barley and rye. All negative ELISA samples (i.e. results below the limit of quantification of the respective ELISA, namely 5 mg/kg gluten for the RIDASCREEN® Gliadin and 10 mg/kg gluten for the RIDASCREEN®FAST Gliadin) showed also negative results in the RIDA®QUICK Gliadin.

All samples with ELISA results > 5 mg/kg gluten were also detected positive with RIDA®QUICK Gliadin. It is important to note that the sensitivity might differ for different sample matrices. The results of the RIDA®QUICK Gliadin correlate very well with the results of the ELISA.

Table 7: Samples extracted with ethanol and analysed by RIDASCREEN® ELISAs and the immunochromatographic RIDA®QUICK Gliadin

Sample	Description	RIDASCREEN®Gliadin mg/kg gluten	RIDASCREEN®FAST Gliadin mg/kg gluten	RIDA®QUICK Gliadin*
Noodles from maize	contaminated	6.2	< 10	+
Casein (Sigma)	negative	< 5	< 10	–
Mixture of spices	contaminated	7.2	< 10	+
Maize flour	negative	< 5	< 10	–
Flour of rice 02	negative	< 5	< 10	–
Spaghetti	gluten free (< 20 mg/kg)	15.4	18.0	+
Apple fiber	negative	< 5	< 10	–
Buckwheat grits	negative	< 5	< 10	–
Skim milk powder	negative	< 5	< 10	–
Oats, grinded the whole grain	contaminated	2596	3614	+
Wheat starch	contaminated	56.6	46.6	+
Rusk	contaminated	49.2	35.0	+
Waffle bread 01	contaminated	15.8	14.8	+
Flour of rice 02	neg. reference	< 5	< 10	–
Flour of rice	contaminated	207	188.8	+
Flour of maize 2	contaminated	1107	1459	+
Flour of maize 1	contaminated	70.2	56.4	+
Flour of maize 3	negative	< 5	< 10	–
Flour of maize 574	contaminated	53.2	32.6	+
Flour of maize	contaminated	94.0	78.8	+
Flour not specified	contaminated	13.4	17.0	+

* RIDA®QUICK Gliadin results are indicated:
"–" no red line visible
"+" red line visible

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Heat treated samples should be extracted with Cocktail (patented). During the baking process most of the alpha, beta and gamma monomeric gliadins are denatured as aggregated insoluble forms and cannot be extracted with 60 % ethanol. Consequently, gluten values in heat-processed foods, when extracting only with ethanol are always underestimated. Therefore, a gluten extraction procedure for heat-processed foods was developed using the Cocktail (patented) to renaturate the monomeric forms. Table 8 shows bread samples extracted with the Cocktail (patented). The bread samples have been prepared by adding various amounts of purified WGPAT material to the bread dough before baking. A good correlation was found for the dip stick RIDA®QUICK Gliadin with the RIDASCREEN® ELISAs.

Table 8: Cocktail (patented) extraction of several spiked breads, analysed by RIDASCREEN® ELISAs and RIDA®QUICK Gliadin

Sample	Declared	RIDASCREEN®Gliadin mg/kg gluten	RIDASCREEN®FAST Gliadin mg/kg gluten	RIDA®QUICK Gliadin*
Bread	278	253.4	256.0	+
Bread	154	149.6	158.2	+
Bread	66	67.0	64.8	+

"–" no red line visible
"+" weak red line visible

In the collaborative study of the Working Group on Prolamin Analysis and Toxicity (WGPAT) the RIDASCREEN® Gliadin (R7001) was tested by 20 international laboratories in the year 2000. The 12 samples analyzed were partly raw materials and partly processed food with concentrations between 0 and 400 mg/kg gluten. As far as the processed samples are concerned the dough was spiked with PWG gliadin before baking the bread (factor 2 was used to calculate from gliadin to gluten content). For the sample preparation all samples were extracted with Cocktail (patented). The dip stick RIDA®QUICK Gliadin showed a good correlation with the ELISA results as can be seen in Table 9.

Table 9: RIDASCREEN® results (mg/kg gluten) of the WGPAT ring trial samples. All samples were extracted with Cocktail (patented).

Sample	Declared	RIDASCREEN®Gliadin mg/kg gluten	RIDASCREEN®FAST Gliadin mg/kg gluten	RIDA®QUICK Gliadin*
Heat treated				
Maize + PWG gliadin	336	296	346	+
Maize + PWG gliadin	68	64	58	+
Maize + PWG gliadin	160	150	158	+
Maize	contaminated	12	14	+
Not heat treated				
Rice + PWG glidain	82	60	64	+
Rice	negative	< 5	< 10	-
Wheat starch	28	16	14	+
Rice flour	26	30	36	+
Maize flour I	negative	< 5	< 10	-
Maize flour II	negative	< 5	< 10	-

* RIDA®QUICK Gliadin results are indicated:
"–" no red line visible
"+" weak red line visible

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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).

Real time stability of the test will regularly be controlled according to the total quality management schedule of the company.

Limitations

For evaluation of different foods only one exemplary sample was analyzed, other samples may show a different result. Due to the multitude of food types, matrix effects cannot be excluded. In processed food, proteins may be altered or fragmented; this may have an impact on the recovery. Cleansing reagents containing hypochlorite disturb the test. It is assumed that the gliadin proteins get oxidized by hypochlorite and are no longer detectable. In order to ensure a high analytical performance:

- Use also gluten free and gluten containing (spiked) samples as test controls (e.g. Art. No. R7012)
- Carry out spiking experiments (see appendix 1 of the document R5 Gluten product line – specificity, cross reactivities, extraction efficiency)
- Confirm results with PCR (e.g. SureFood® ALLERGEN Gluten, Art. No. S3606)

In the case of swabbing test bands with non–uniform intensity may occur due to an inhomogeneous gluten distribution on the surface or different swabbing procedures.

To investigate the **hook-effect** (false negative result in highly contaminated samples due to an overload of antibodies by the antigen) different kinds and concentrations of wheat, rye and barley were analyzed. When testing pure wheat, rye and barley a red test band was visible. However, this test band was slightly blurred. From table 3 it can be concluded that no hook-effect is observed for high concentrated wheat, rye and barley samples.

Table 10: Analysis of high concentrated wheat, rye and barley samples with RIDA®QUICK Gliadin to investigate the hook-effect. The extraction was carried out with Cocktail (patented) (Art. No. R7006).

Sample	Cereal concentration in %	Result*
Wheat “Type 405“	100	+
	50	+
	25	+
Wheat “Ritmo”	100	+
	50	+
	25	+
	12.5	+
	6.25	+
Rye “Nikita”	100	+
Barley “Allegra”	100	+

* RIDA®QUICK Gliadin results are indicated:
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"+" red line visible

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Conclusion

RIDA®QUICK Gliadin is a reliable test system for the analysis of food and cleansing water as well as for carrying out swabs for hygiene control. With the RIDA®QUICK Gliadin it is possible to distinguish between gluten-free and gluten contaminated samples at very low gluten levels. Positive screening results should be quantified using ELISA RIDASCREEN® Gliadin (Art. No. R7001) or RIDASCREEN®FAST Gliadin (Art. No. R7002) or RIDASCREEN®FAST Gliadin sensitive (R7051).

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in New Zealand please contact
Bio-Strategy Limited
T: 0800 34 24 66 | E: sales.nz@bio-strategy.com
www.bio-strategy.com | shop.bio-strategy.com

For further information or orders please contact R-Biopharm AG:

International Sales:
Phone: +49 (0) 61 51 - 81 02-0
Fax: +49 (0) 61 51 - 81 02-40
E-mail: *sales@r-biopharm.de*

Order Department:
Phone: +49 (0) 61 51 - 81 02-0
Fax: +49 (0) 61 51 - 81 02-20
E-mail: *orders@r-biopharm.de*

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