

● Introduction to Gluten-free Food

Coeliac disease is a genetically determined disorder in which there is an abnormal response to ingested cereal proteins. There is an over exuberant immunological reaction to prolamins from wheat, barley and rye resulting in a mucosa damage of the small

intestine. The only treatment is adherence to a gluten-free or reduced-gluten diet for life. Avoiding gluten in the diet is not easy since cereals are used in the preparation of many foods.

● Codex Alimentarius Standard for Gluten-free Food

The Codex Standard (CODEX STAN 118-1979) defines limit values:

a) Dietary foods consisting of or made only from one or more ingredients which do not contain wheat (i.e., all Triticum species, such as durum wheat, spelt, and kamut), rye, barley, oats or their crossbred varieties, and the **gluten level does not exceed 20 mg/kg** in total, based on the food as sold or distributed to the consumer, and/or

b) Dietary foods consisting of one or more ingredients from wheat (i.e., all Triticum species, such as durum wheat, spelt, and kamut), rye, barley, oats or their crossbred varieties, which have been specially processed to remove gluten, and the **gluten level does not exceed 20 mg/kg** in total, based on the food as sold or distributed to the consumer.

● The Mendez ELISA R5 Method

Recent research has focused on the R5 sandwich ELISA procedure developed by Mendez and co-workers. The R5 ELISA has been collaboratively tested and was endorsed as type I method by the CODEX Alimentarius.

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

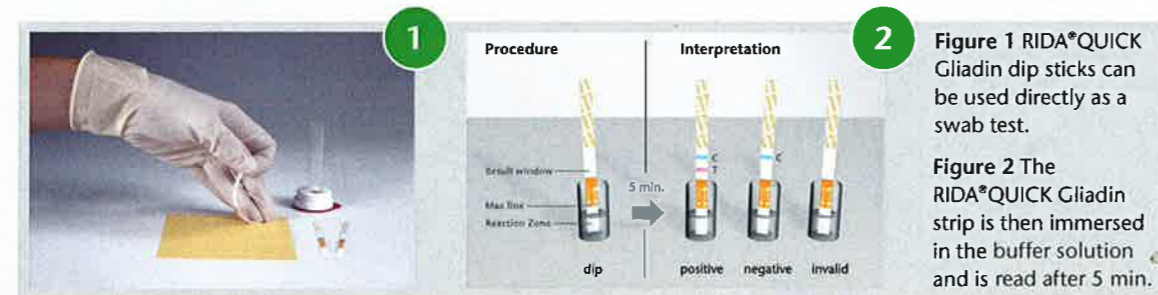
is also a Performance Tested Method of the AOAC Research Institute (AOAC-RI 120601).

It is important to use the Cocktail (patented) (R7006) or the RIDA® Extraction Solution (R7099) for the extraction procedure as it improves the response to heat-treated prolamins. The R5 method has equal reactivity towards the prolamins of wheat, rye and barley, with no reported interference from soy, oats, corn (maize) etc.

● Food-safety management: Hygiene control and product testing

A Hazard Analysis Critical Control Point (HACCP) is necessary to ensure gluten-free products. RIDA®QUICK Gliadin (R7003) is an ideal tool for carrying out surface swabs

for hygiene control as can be seen in Figure 1. The test system is very user friendly and qualitative results are available within 5 min (see Figure 2).



● Test Kits – Dip Stick or Competitive / Sandwich ELISA

Depending on the food processing different test kits are recommended for gliadin analysis as can be seen in Table 1. Raw materials that were surely not heat treated can be simply extracted with ethanol and then analysed within 5 minutes with the dip stick RIDA®QUICK Gliadin (Art. No. R7003/R7004). Gliadin in biscuits, bread, choco-

late and cereals should be determined with the sandwich ELISA RIDASCREEN® Gliadin (Art. No. R7001/R7002). For the analysis of fermented and hydrolyzed food (e.g. beer, starch syrup, starch, malt extract, sourdough, soy sauce) the RIDASCREEN® Gliadin competitive (Art. No. R7021) should be used.

Table 1 Different processed food and the respective gliadin test kits recommended for analysis

Intended Use	Raw materials (e.g. maize flour) and surfaces (swabs for hygiene control)	All kinds of food samples (e.g. biscuits, bread, chocolate)		Hydrolyzed and fermented food (e.g. beer, starch, malt extract)
Test Kit	RIDA®QUICK Gliadin (Art. No. R7003/R7004)	RIDASCREEN® Gliadin (Art. No. R7001)	RIDASCREEN®FAST Gliadin (Art. No. R7002)	RIDASCREEN® Gliadin competitive (Art. No. R7021)
Method	Immunochromatographic dip stick (qualitative)	Sandwich ELISA (quantitative)	Sandwich ELISA (quantitative)	Competitive ELISA (quantitative)
Antibody used	R5-mAb	R5-mAb	R5-mAb	R5-mAb
Calibrator Material	No calibrator	Calibrated to the purified gliadin material of the WG PAT		Prolamin hydrolysate (mixture of wheat, rye and barley)
Extraction	Ethanol (60 %)	Cocktail (patented) (Art. No. R7006) or RIDA® Extraction Solution (Art. No. R7099)		Ethanol (60 %) or Ethanol-fish-gelatine
Detection	Intact prolamins and large fragments with more than one binding site			Small peptide fragments and intact prolamins
Detection Limit	2.5 mg/kg gliadin	1.5 mg/kg gliadin	2 mg/kg gliadin	1.4 mg/kg gliadin
Limit of Quantitation	(Qualitative test)	2.5 mg/kg gliadin	5 mg/kg gliadin	5 mg/kg gliadin
Incubation time	5 min	90 min	30 min	40 min
Validation	Internal	AOAC-RI certified, international collaborative study (WG PAT), collaborative study is in progress	Internal	Internal, collaborative study is in progress

● Results of Surveillance of Gluten-free Food

Gluten-free products are constantly surveyed for the presence of gluten by the manufacturers or by food inspectors. Corn (maize) and soy products (naturally gluten-free grains) are often significantly

contaminated. In table 2 SureFood® Gluten has been successfully used as a confirmatory method for a wheat contamination of two different soy cheese samples.

Table 2 Soy cheese contamination with wheat

Food	RIDASCREEN® Gliadin ELISA [Gliadin mg/kg]	SureFood® PCR Gluten	PCR Wheat
Soy cheese 1 (gluten-free)	49.9	positive	positive
Soy cheese 2 (gluten-free)	39.6	positive	positive



R-Biopharm's Product Portfolio for Gluten Analysis

Product	Description	No. of Tests/Amount	Art. No.
RIDASCREEN® Gliadin AOAC-OMA (2012.01) AOAC-RI (120601) Codex Alimentarius Method (Type I)	Official R5 Mendez method: Sandwich ELISA to quantify prolamines from wheat, rye and barley in e.g. food declared as gluten-free. Sample extraction with R7006, R7016 or R7098, R7099 (not contained in the kit). Detection limit: 1.5 mg/kg (ppm) gliadin or 3.0 mg/kg (ppm) gluten	96 determinations Incubation time: 1 h 30 min	R7001
RIDASCREEN®FAST Gliadin	R5 sandwich ELISA to quantify prolamines from wheat, rye and barley in e.g. food declared as gluten-free. Sample extraction with R7006, R7016 or R7098, R7099 (not contained in the kit). Detection limit: 2.0 mg/kg (ppm) gliadin or 4.0 mg/kg (ppm) gluten	48 determinations Incubation time: 30 min	R7002
RIDA®QUICK Gliadin	The immunochromatographic test is based on the R5 antibody and detects prolamins from wheat, rye and barley. The strips can be used directly for swabs on surfaces or for analysis of e.g. gluten-free raw materials. Detection limit: 0.5 µg gliadin/100 cm² on surfaces. Detection limit: 2.5 mg/kg (ppm) gliadin in e.g. gluten-free raw materials	25 test strips in a reclosable tube, 25 plastic pipettes, sample diluent (concentrate), 30 vials Incubation time: 5 min	R7003
New! RIDA®QUICK Gliadin (single packaged)	Corresponding to R7003	25 test strips single packaged, sample diluent (ready-to-use), 30 vials Incubation time: 5 min	R7004
New! RIDASCREEN® Gliadin competitive 2 nd Generation	R5 competitive ELISA to quantify potential toxic peptide sequences from prolamins from wheat, rye and barley in fermented and hydrolyzed food (e.g. beer, starch, starch syrup, malt extracts). The standard material is a hydrolyzate (mixture of wheat, rye and barley); the results can be related to the gluten limit values of the Codex Alimentarius. Detection limit: 1.36 mg/kg (ppm) gliadin or 2.72 mg/kg (ppm) gluten	96 determinations Incubation time: 40 min	R7021
Cocktail (patented)	Developed by Prof. Mendez; officially recommended extraction buffer for all processed e.g. heat treated food samples in conjunction with R7001, R7002, R7003, R7004.	105 ml 1000 ml	R7006 R7016
New! RIDA® Extraction Solution (colorless)	Alternative to the Cocktail (patented): The extraction is faster (35 min compared to 1 h 50 min with the Cocktail). It is used in combination with R7001, R7002, R7003, R7004 and R4612 (casein extraction of bakery goods and sausages).	105 ml	R7098
RIDA® Extraction Solution / Extraktionslösung	Corresponding to R7098 but stained red to simplify ELISA pipetting	105 ml	R7099
Set of 3 Gliadin Assay Controls	Three control materials: 1 negative, 2 positive (homogenized flour samples)	3 x 1.5 g	R7010
SureFood® ALLERGEN real time PCR Gluten	The PCR test is used for screening or as a confirmatory method for the ELISA. The test is specific for wheat, spelt, kamut, barley, rye and oats.	100 reactions	S3106
New! SureFood® ALLERGEN QUANT real time PCR Gluten	Quantitative DNA detection Detection limit: < 5 DNA copies, 0.4 mg/kg depending on matrix and DNA preparation.	100 reactions	S3206
New! SureFood® QUANTARD Allergen 40 Laboratory reference material for quantitative real-time PCR	Corn flour contains 14 potential allergens in food except sulfite and lactose with a concentration of 40 mg/kg. Material is developed for quantification of allergens in food.	2 g	S3301

Analytical Test Kits to ensure Gluten-free Food

R-Biopharm offers a whole range of ready to use test systems for gliadin/gluten analysis.

Surface and hygiene control

Gluten-free production conditions are a prerequisite for gluten-free products. Therefore, swabs within production sites should be carried out regularly with RIDA®QUICK Gliadin.

Product testing

RIDASCREEN® ELISAs allow gluten quantification to ensure compliance with legal limit values of 20 mg/kg for gluten-free food.

All immuno test systems

- are based on the R5 antibody
- detect prolamins from wheat, rye and barley
- show no cross-reactivity to soy, oats, corn, etc.
- are ideal to comply with legal requirements of the Codex Alimentarius

Additionally, R-Biopharm offers

- the Cocktail (patented) or the RIDA® Extraction Solution for sample extraction
- Gliadin Assay Controls

Lateral Flow

RIDA®QUICK Gliadin Swabbing

ELISA

RIDASCREEN® Gliadin

- AOAC-OMA (2012.01)
- AOAC-RI (120601)
- Codex Alimentarius Method (Type I)

PCR

SureFood® Gluten

R-Biopharm – for the safety of your analysis.