T: 02 8212 4074 F: 02 9423 6992
info@keydiagnostics.com.au
www.keydiagnostics.com.au
PO Box 1038, Gymea, NSW, 2227

RIDA®QUICK Gliadin

Art. No. R7003

Immunchromatographischer Test zum Nachweis von Gluten auf Oberflächen, in Lebensmitteln und in Reinigungs-/Prozesswasser

Immunochromatographic test for the detection of gluten on surfaces, in food, and in cleansing / process water

Geprüft als / approved as
AOAC *Official Methods*℠ (2015.16)
for processed/non-processed corn products

AOAC *Performance Tested Methods*℠ (101702)
for surfaces and cleansing waters

PERFORMANCE TESTED
AOAC
RESEARCH INSTITUTE
LICENSE NUMBER 101702

In vitro Test

Lagerung bei 2 - 8 °C
Storage at 2 - 8 °C

RIDA®QUICK Gliadin

Brief information

RIDA®QUICK Gliadin (Art. No. R7003) is an immunochromatographic test for the qualitative detection of gluten contamination
- on surfaces (swab test for the hygiene control in production and in laboratories)
- in cleansing waters (CIP waters)
- in gluten-free raw material after ethanol extraction
- in gluten-free processed food after extraction with the Cocktail (patented) or with RIDA® Cocktail ECO.

The R5 dip stick RIDA®QUICK Gliadin has been approved as AOAC-OMA 2015.16 for corn based food matrices using Cocktail (patented) or ethanol extraction and AOAC PTM (101702) for swabbing and cleansing waters.

The test kit contains 25 test strips (in a tube) for 1 determination each. All reagents required for the swab test are contained in the test kit. Results are evaluated visually.

Time requirement:

sampling for swab test	approx. 1 min
sample preparation	
for 10 cleansing waters	approx. 5 min
for 10 raw materials	approx. 15 min
for processed food (R7006)	approx. 120 min
for processed food (R7080)	approx. 35 min
test implementation (incubation time)	5 min

Detection limit:
- **surfaces** approx. 1.6 - 3 µg gluten / 100 cm^2
- **raw material** approx. 4.4 mg/kg gluten depending on food matrix
- **processed food** approx. 6.3 mg/kg gluten depending on food matrix
- **cleansing water (without cleansing reagent)** approx. 10 ng/ml gluten
- **cleansing water (with cleansing reagent)** approx. 50 - 100 ng/ml gluten

Specificity: The **monoclonal antibody R5** reacts with the gliadin-fraction from wheat and corresponding prolamins from rye and barley.

Cross reactivities of the used antibody have been determined for the pure food (e.g. corn flour). In a composed / processed food (e.g. maize bread) cross reactivities might be different. Interfering substances (e.g. polyphenols) can be detected by spike experiments.

Related products:
RIDASCREEN® Gliadin (Art. No. R7001)
RIDASCREEN®FAST Gliadin (Art. No. R7002)
RIDASCREEN®FAST Gliadin sensitive (Art. No. R7051)
RIDASCREEN® Gliadin competitive (Art. No. R7021)
RIDA®QUICK Gliadin (single packaged) (Art. No. R7004)
RIDA®QUICK Gliadin (ready to swab) (Art. No. R7005)
Cocktail (patented) (Art. No. R7006 / R7016)
RIDA® Cocktail ECO (Art. No. R7080)
RIDA® Extraction Solution (colorless) (Art. No. R7098)
Set of 3 processed Gliadin Assay Controls (Art. No. R7012)
SureFood® ALLERGEN PCR Gluten (Art. No. S3606)

1. Intended use

RIDA®QUICK Gliadin can be used for gluten detection on surfaces for hygiene control (swab test), in cleansing waters, and in raw material and processed food. 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 very high gluten concentrations (> 10000 mg/kg gluten).

2. General

The use of wheat flour and gluten in foodstuff is extremely common because of their useful effects on e.g. texture, moisture retention and flavour. Gluten is a mixture of prolamin and glutelin proteins present in wheat, rye and barley.

Coeliac disease is a permanent intolerance to gluten that results in damage to the small intestine and is reversible when gluten is avoided by diet.

The Codex Alimentarius Commission has stipulated in the „Codex Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten" (CODEX STAN 118-1979) the limit value for gluten-free food at 20 mg/kg gluten. This threshold has also been adopted by many national legislations. The prolamin content (e.g. gliadin) of gluten is generally assumed to be 50 % (CODEX STAN 118-1979).

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 RIDASCREEN® Gliadin test (Art. Nr. R7001). **The RIDA®QUICK Gliadin test strips also use the R5 antibody and show a good correlation with the official method, the R5-ELISA RIDASCREEN® Gliadin. R-Biopharm AG is the only company that is allowed to use the R5 antibody for test strips.**

3. Test principle

The basis of the immuno-chromatographic test is the monoclonal R5-antibody which is specific for the detection of gliadin from wheat and prolamins from rye and barley. If gliadin is present, a sandwich is formed at the test band consisting of immobilized R5 antibody at the target band, gliadin and red latex-labeled R5 antibody.
Results are read visually. Generally, the higher the analyte level in the sample, the stronger the red color of the test band will be.

4. Reagents provided

Each kit contains sufficient materials for 25 measurements.

Component	Cap color	Format		Volume
Test strip	-	Ready to use		25 pieces
Test tube				30 pieces
Disposable pipet				25 pieces
Buffer	transparent	Ready to use		60 ml
Evaluation card				1 piece

5. Materials required but not provided

5.1. Equipment:

For analysis of raw material and processed food

- scales
- laboratory mincer / grinder, pestle and mortar, Ultra-Turrax or homogenisator
- shaker
- centrifugal vials + centrifuge or paper filter
- graduated pipettes

5.2. Reagents:

For analysis of raw material

- distilled or deionized water
- skimmed milk powder (food quality) for soy, tannin and polyphenol containing food
- ethanol solution (60 %), for the extraction of the samples (add 150 ml ethanol p.a. to 100 ml distilled water and shake well)

For analysis of processed food

- distilled or deionized water
- skimmed milk powder (food quality) for tannin, and polyphenol containing food
- ethanol solution (80 %), for the extraction of the samples (add 120 ml ethanol p.a. to 30 ml distilled water and shake well)
- Cocktail (patented) (Art. No. R7006 / R7016) or RIDA® Cocktail ECO (R7080)

6. Warnings and precautions for the users

This test should be carried out by trained staff only. The instructions for use must be strictly followed.

This kit may contain hazardous substances. For hazard notes on the contained substances please refer to the appropriate material safety data sheets (MSDS) for this product, available online at www.r-biopharm.com.

7. Storage instructions

Store the unopened kit at 2 - 8 °C (36 - 46 °F). Do not freeze the kit.

Once the test strip container has been opened, store the container at room temperature (20 - 25 °C / 68 - 77 °F).

The dip sticks are very sensitive to humidity that could turn the test useless. For this reason keep the strips away from humidity!

No quality guarantee is furnished after the expiry date on the kit label.

8. Test procedure

Airborne dust and dirty laboratory equipment lead to gluten contamination of the assay. In order to avoid cross-contamination by cereal dust, please note the following points:

- wear gloves before starting and during the assay
- clean surfaces, glass vials, mincers and other equipment with 40 % ethanol or 2-propanol
- for the analysis, the extraction and the test procedure should be carried out in separate rooms
- when using the Cocktail (patented), it is recommended to work **under a chemical hood,** because it contains ß-mercaptoethanol

8.1. Swab test: sampling and test implementation

For the AOAC-RI validation stainless steel, sealed ceramic, plastic and silicone rubber were validated (see validation report).

1. Take as many test tubes as surfaces to be analyzed.
2. Place 500 µl of buffer in the test tube (e.g. using the disposable pipette provided).
3. Swab the lower end (reaction zone) of a dry dip stick thoroughly over a sampling area of 10 x 10 cm (wear gloves).

4. Place the dip stick vertically into the test tube with the arrow pointing down. Do not immerse the dip stick beyond the maximum line.
5. Take out the strip after exactly 5 min (+/- 10 s) and read the result using the evaluation card.

8.2. Analysis of cleansing water (CIP water)

For the AOAC-RI validation commercial cleansing solutions and pure water were validated (see validation report).

8.2.1 Cleansing water **without** detergent

1. Take as many test tubes as samples to be analyzed.
2. Place 250 µl of buffer in the test tube (e.g. using the disposable pipette provided).
3. Place 250 µl of cleansing water in the test tube (e.g. using the disposable pipette provided) and mix gently
4. Place the dip stick vertically into the test tube with the arrow pointing down. Do not immerse the dip stick beyond the maximum line.
5. Take out the strip after exactly 5 min (+/- 10 s) and read the result using the evaluation card.

8.2.2 Cleansing water **with** detergent

1. Take as many test tubes as samples to be analyzed.
2. Place 500 µl of buffer in the test tube (e.g. using the disposable pipette provided).
3. Place 50 µl of cleansing water in the test tube (e.g. using the disposable pipette provided) and mix gently
4. Place the dip stick vertically into the test tube with the arrow pointing down. Do not immerse the dip stick beyond the maximum line.
5. Take out the strip after exactly 5 min (+/- 10 s) and read the result using the evaluation card.

8.3. Analysis of food samples

For AOAC OMA (2015.16) validation processed and non-processed corn samples using ethanol and Cocktail (patented) extraction were analyzed (see validation report).

Addition of skimmed milk powder to the sample preparation
Depending on the extraction method, the addition of skimmed milk powder is necessary for some ingredients to avoid unwanted disturbing reactions.

Food ingredient	Ethanol extraction	Cocktail (patented) / RIDA® Cocktail ECO extraction
Soja	1 g skimmed milk powder	—
Tannin- and polyphenol containing food (e.g. chocolate, coffee, cacao, chestnut flour, buckwheat, millet and spices)	1 g skimmed milk powder	0.25 g skimmed milk powder

8.3.1. Extraction with ethanol for raw material (fluid and soft non processed material)

- **fluid raw material:** mix 1 ml of the sample with 9 ml 60 % ethanol solution
- for soy milk/tannin- and polyphenol containing food add additionally 1 g of skimmed milk powder
- **soft raw material:** weigh 1 g of a representative sample and add 10 ml 60 % ethanol solution
- for soy milk/tannin- and polyphenol containing food add additionally 1 g of skimmed milk powder
- shake well for at least 30 sec. (vortex)
- centrifuge: 10 min / at least 2500 g / room temperature (20 - 25 °C / 68 - 77 °F)
- alternatively: let the sample settle down and / or filtrate

8.3.2. Extraction with ethanol for raw material (solid and hard non processed raw material)

- weigh 5 g sample and grind it to powder
- use 1 g of this powder and add 10 ml 60 % ethanol solution (for soy /tannin- and polyphenol containing food add 1 g of skimmed milk powder)
- shake well for at least 30 sec. (vortex)
- centrifuge: 10 min / at least 2500 g / room temperature (20 - 25 °C / 68 - 77 °F)
- alternatively: let the sample settle down and / or filtrate

8.3.3 Extraction with Cocktail (patented) for processed food

Homogenize well a sufficient amount (at least 50 g or 50 ml) of sample (grind it thoroughly to powder and mix well or mix well the solution respectively).

- **liquid food samples**: use 0.25 ml of the homogenized sample (with tannin and polyphenol containing samples add 0.25 g of skimmed milk powder) and add 2.5 ml of the Cocktail (patented), close the vial and mix well

- **other food samples (e.g. soya / quinoa containing food):** to 0.25 g of a homogenized sample add 2.5 ml of Cocktail (patented), close the vial and mix well
- **tannin and polyphenol containing food samples (e.g. chocolate, coffee, cocoa, chestnut flour, buckwheat, millet and spices)**: weigh 0.25 g of the homogenized sample, add 0.25 g of skimmed milk powder and add 2.5 ml of the Cocktail (patented), close the vial and mix well
- **meat and sausages:** in these matrices gluten may be distributed not evenly; therefore, weigh 50 g sample and homogenize: weigh 0.25 g of the homogenized sample and add 2.5 ml of the Cocktail (patented), close the vial and mix well
- **oat samples:** gluten may not distributed evenly; furthermore the samples are difficult to homogenize. Therefore, 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), close the vial and mix well

Please further extract all samples as described in the following:

- incubate for 40 min at 50 °C (122 °F) in the water bath
- let the sample cool down and then mix it with 7.5 ml 80 % ethanol (see 5.2.) (for oat samples: 30 ml 80% ethanol)
- close the vial and shake for 1 h upside-down or by a rotator at room temperature (20 - 25 °C / 68 - 77 °F)
- centrifuge: 10 min, at least 2500 g, at room temperature (20 - 25 °C / 68 - 77 °F) or 2 ml of the extract can be centrifuged with high speed for 10 min in reaction caps by using a microcentrifuge to obtain a particle free supernatant (alternatively, the extract can only be filtered)
- put the particle free supernatant in a screw top vial (depending on the sample the supernatant needs to be filtered too)

Remark: All supernatants / filtrates obtained after centrifugation or filtration can be stored in a tightly closed vial in the dark at room temperature (20 - 25 °C / 68 - 77 °C) up to four weeks.

8.3.4 Extraction with RIDA® Cocktail ECO for processed food

The faster sample preparation using the environmental-friendly **Cocktail ECO** (R7080) is convenient for the screening of samples. The Cocktail ECO has an extraction efficiency of approx. 70 - 110% compared to Cocktail (patented).

Homogenize well a sufficient amount (at least 50 g or 50 ml) of sample (grind it thoroughly to powder and mix well or mix well the solution respectively). Prepare the necessary amount of RIDA® Cocktail ECO according to the product information R7080.

- **liquid food samples**: use 0.25 ml of the homogenized sample (with tannin and polyphenol containing samples add 0.25 g of skimmed milk powder) and add 2.5 ml of RIDA® Cocktail ECO, close the vial and mix well
- **other food samples (e.g. soya and quinoa containing food):** to 0.25 g of a homogenized sample add 2.5 ml of RIDA® Cocktail ECO, close the vial and mix well
- **tannin and polyphenol containing food samples (e.g. chocolate, coffee, cocoa, chestnut flour, buckwheat, millet and spices)**: weigh 0.25 g of the homogenized sample, add 0.25 g of skimmed milk powder and add 2.5 ml of RIDA® Cocktail ECO, close the vial and mix well
- **meat and sausages:** in these matrices gluten may be distributed not evenly; therefore, weigh 50 g sample and homogenize: weigh 0.25 g of the homogenized sample and add 2.5 ml of the RIDA® Cocktail ECO, close the vial and mix well
- **oat samples:** gluten may not be distributed evenly; furthermore the samples are difficult to homogenize. Therefore, 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 RIDA® Cocktail ECO, close the vial and mix well

Please further extract all samples as described in the following:

- incubate for 10 min at 50 °C (122 °F) in the water bath
- let the sample cool down and then mix it with 7.5 ml 80 % ethanol (see 5.2.) (for oat samples: 30 ml 80% ethanol)
- close the vial and shake for 10 min upside-down or by a rotator at room temperature (20 - 25 °C / 68 - 77 °F)
- centrifuge: 5 min, at least 2500 g, at room temperature (20 - 25 °C / 68 - 77 °F) or 2 ml of the extract can be centrifuged with high speed for 5 min in reaction caps by using a microcentrifuge to obtain a particle free supernatant (alternatively, the extract can only be filtered)
- put the particle free supernatant in a screw top vial (depending on the sample the supernatant needs to be filtered too)

Remark: All supernatants / filtrates obtained after centrifugation or filtration can be stored in a tightly closed vial in the dark at room temperature (20 - 25 °C / 68 - 77 °C) up to two weeks.

8.3.5 Test implementation for raw material and processed food

1. Take as many test tubes as samples to be analyzed.
2. Place 500 µl of buffer in the test tube (e.g. using the disposable pipette provided).
3. Pipette 50 µl of the sample supernatant / filtrate or place 3 drops with the provided disposable pipette, vertically dropped in the test tube and shake slightly.
4. Place the dip stick vertically into the test tube with the arrow pointing down. Do not immerse the strip beyond the maximum line.
5. Take out the dip stick after exactly 5 min (+/- 10 s) and read the result using the evaluation card.

9. Results

Positive result: two colored bands

The sample is positive if two colored bands (the blue control band and the red test band) are visible within the result window. In the case of swabbing complete test bands with non-uniform intensity may occur due to an inhomogeneous gluten distribution on the surface or different swabbing procedures.

Swab test:	> 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

Negative result: only the blue control band

The sample is negative if no red test band is visible within the result window.

Swab test:	< 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

Invalid result: no colored band

If no band is visible within the result window after performing the test or if an incomplete test band is visible, the test is considered invalid.

In general

Samples tested negative still may contain gluten contamination below the limit of detection of the assay or other cereal components like starch for example.

Due to the multitude of food types, matrix effects cannot be excluded. In processed food (e.g. heat treatment, dehydration, etc.), proteins may be altered or fragmented, this may have an impact on the recovery/cross reactivity.

For evaluation of the cross reactivity only one exemplary sample was analyzed, other samples may show a different result. All cross-reactivities and exemplary analysed matrices are described in the updated validation report.

The test strip has been developed for the detection of gluten contamination.

The limit of detection depends on sample type and extraction efficiency or the properties of the swabbed surface and the kind of contamination respectively.

The sample extraction with ethanol should only be used for raw material that were surely not heated and not processed.

A negative result does not necessarily indicate the absence of gluten as gluten may be not homogenously distributed or the level of gluten in the product is below the limit of detection.

Limitations

Cleansing water containing hypochlorite cannot be analyzed. This cleaning agent destroys gluten very quickly in the sample by oxidation. The test strip is not able to detect potentially remaining gluten fragments.

Recommendations

In order to ensure a high analytical performance it is recommended to

- adjust the pH to a neutral value for extremely acidic or alkaline samples
- use assay test controls (R7012, for cocktail extraction) or spiked samples for quality control
- carry out spiking experiments for an accurate and correct procedure
- compare the extraction efficiency of ethanol and RIDA® Cocktail ECO (R7080) with the Cocktail (patented) (R7006/R7016)
- use RIDASCREEN® Gliadin (Art. No. R7001) for quantification, this test kit is also AOAC-RI and AOAC-OMA (Official Method of Analysis) validated
- perform SureFood® PCR to confirm results

For documentation, the upper part of the dip stick marked with “Gluten” together with the test bands must be cut off.

Further information, validation data and applications are available on request from your local distributor or R-Biopharm AG.

Further applications:

- Sample preparation for processed food with the RIDA® Extraction Solution (colorless) (Art. No. R7098) - **only after validation**
- Sample preparation for polyphenol containing raw material using fish gelatine

The data corresponds to our present state of technology and provides information on our products and their uses. R-Biopharm makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Defective products will be replaced. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. R-Biopharm shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

R-Biopharm AG
Postanschrift / Postal Address:
An der neuen Bergstraße 17
64297 Darmstadt, Germany
Sitz / Corporate Seat: Pfungstadt
Tel.: +49 (0) 61 51 - 81 02-0
Fax: +49 (0) 61 51 - 81 02-40
E-mail: info@r-biopharm.de
www.r-biopharm.com