RIDASCREEN® Gliadin

Enzymimmunoassay zur quantitativen Bestimmung von Gliadinen und verwandten Prolaminen

Enzyme immunoassay for the quantitative analysis of gliadins and corresponding prolamines

Art. No.: R7001

PERFORMANCE TESTED
AOAC
RESEARCH INSTITUTE
LICENSE NUMBER 120601

In vitro Test
Lagerung bei 2 - 8 °C
Storage at 2 - 8 °C

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Brief information

RIDASCREEN® Gliadin (R7001) is a sandwich enzyme immunoassay for the quantitative analysis of prolamins from wheat (gliadin), rye (secalin) and barley (hordein) in as gluten-free declared food.

The R5 ELISA RIDASCREEN® Gliadin is
- accepted as AOAC-OMA (2012.01)
- certified at AOAC-RI (120601)
- Codex Alimentarius Method (Type I)

All reagents required for the enzyme immunoassay - including standards - are contained in the test kit. The test kit is sufficient for 96 determinations (including standards). A microtiter plate spectrophotometer is required for quantification.

Sample preparation:	homogenization and extraction
Standard material:	The RIDASCREEN® standard material is calibrated to the standard of the Prolamin Working Group.
Time requirement:	sample preparation (for 10 samples) .....approx. 2 h test implementation (incubation time) .....1.5 h
Detection limit:	1.5 mg/kg (ppm) gliadin or to 3 mg/kg (ppm) gluten
Limit of quantification:	2.5 mg/kg (ppm) gliadin or to 5 mg/kg (ppm) gluten
Recovery rate:	92 - 113 % for different food matrices
Specificity:	The monoclonal antibody R5 reacts with the gliadin-fractions from wheat and corresponding prolamins from rye and barley.
Cross reactivity:	No cross reaction with soy, oats, corn (maize), rice, millet, teff, buckwheat, quinoa and amaranth.

Related products

RIDASCREEN®FAST Gliadin (R7002)
RIDASCREEN®QUICK Gliadin (R7003/R7004)

RIDASCREEN® Gliadin competitive (R7021)
Cocktail (patented) (R7006/R7016)
RIDA® Extraction Solution (colorless) (R7098)
RIDA® Extraction Solution / Extraktions-Lösung (R7099)
Set of 3 Gliadin Assay Controls (R7010)
SureFood® Allergen QUANT Gluten (S3206)
SureFood® Allergen Gluten (S3106)

1. Intended use

RIDASCREEN® Gliadin is a sandwich enzyme immunoassay for the quantitative analysis of contaminations by prolamins from wheat (gliadin), rye (secalin), and barley (hordein) in raw products like flours (buckwheat, rice, corn, oats, teff) and spices as well as in processed food like noodles, ready-to-serve meals, bakery products, sausages, beverages and ice cream.
All samples should be extracted with the Cocktail (patented) (R7006, official R5-Mendez method) or the RIDA® Extraction Solution / Extraktions-Lösung (R7099).

2. General

The use of wheat flour and gluten in foodstuffs is extremely common because of their heat stability and 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.

According to the Codex Alimentarius (Alinorm 08/31/26) two categories for labeling of food according to the gluten content now exist:
1.) Food products which contain less than 20 ppm can be labeled as **"gluten-free"**.
2.) Food products labeled as **"very low gluten"** can have a gluten content above 20 and up to 100 ppm.

3. Test principle

The basis of the test is the antigen-antibody reaction. The wells of the microtiter strips are coated with specific antibodies against gliadins. By adding the standard or sample solution to the wells, present gliadin will bind to the specific capture antibodies. The result is an antibody-antigen-complex. Components not bound by the antibodies are then removed in a washing step. Then antibody conjugated to

peroxidase is added. This antibody-conjugate is bound to the Ab-Ag-complex. An antibody-antigen-antibody (sandwich) complex is formed. Any unbound enzyme conjugate is then removed in a washing step. Enzyme substrate and chromogen are added to the wells and incubated. Bound enzyme conjugate converts the colorless chromogen into a blue product. The addition of the stop reagent leads to a color change from blue to yellow. The measurement is made photometrically at 450 nm. The absorbance is proportional to the gliadin concentration of the sample.

4. Reagents provided

Each kit contains sufficient materials for 96 measurements (including standard analyses). Each test kit contains:

1 x Microtiter plate (12 strips with 8 removable wells each)
coated with antibodies against gliadin
6 x Gliadin Standards, 1.3 ml each
0 ppb (zero standard), 5 ppb, 10 ppb, 20 ppb, 40 ppb, 80 ppb gliadin
in aqueous solution, ready-to-use
1 x Conjugate (1.2 ml) ..... red cap
peroxidase conjugated antibody, concentrate
1 x Substrate (7 ml) ..... green cap
contains urea peroxide
1 x Chromogen (7 ml) ..... blue cap
contains tetramethylbenzidine
1 x Stop solution (14 ml) ..... yellow cap
contains 1 N sulfuric acid
1 x Sample diluent (60 ml) ..... white cap
as a 5x concentrate
1 x Washing buffer (100 ml) ..... brown cap
10x concentrate

5. Reagents required but not provided

5.1. Equipment

- microtiter plate spectrophotometer (450 nm)
- centrifuge, centrifugal glass vials (e.g. Brand 10742512)
- shaker
- laboratory mincer / grinder, pestle and mortar, ultra-turrax or homogenizer

– water bath (50 °C / 122 °F)
– graduated pipettes
– variable 20 µl - 200 µl and 200 - 1000 µl micropipettes

5.2. Reagents

– distilled or deionized water
– skim milk powder (food quality)
– **Cocktail (patented)** (R7006, 105 ml) and **ethanol solution (80 %)**: i.e. add 120 ml ethanol p.a. to 30 ml distilled water and shake well

or

– **RIDA® Extraction Solution / Extraktions-Lösung** (R7099, 105 ml) and **2-propanol solution (68 %)**: i.e. add 68 ml 2-propanol p.a. to 32 ml distilled water and shake well

6. Warnings and precautions for the users

The stop solution contains 1 N sulfuric acid (R36/38, S2-26).

The buffer contains thimerosal. Avoid contact of the reagent with the skin.

7. Storage instructions

Store the kit at 2 - 8 °C (35 - 46 °F). Do not freeze any test kit components.

Return any unused microwells to their original foil bag, reseal them together with the desiccant provided and further store at 2 - 8 °C (35 - 46 °F).

The colorless chromogen is light sensitive, therefore, avoid exposure to direct light.

No quality guarantee is accepted after expiry of the kit (see kit label).

Do not interchange individual reagents between kits of different lot numbers.

8. Indication of instability or deterioration of reagents

– any coloration of the chromogen solution prior to test implementation
– a value of less than 0.6 absorbance units ($A_{450\ nm} < 0.6$) for standard 6

9. Preparation of Samples

9.1. Preliminary comments

Airborne cereal dust and dirty laboratory equipment lead to gliadin contamination of the assay. Therefore, before starting and during the assay wear gloves.

- clean surfaces, glass vials, mincers and other equipment with 40 % ethanol or 2-propanol (see 5.2.)
- carry out the sample preparation in a room isolated from the ELISA procedure
- check for gliadin contamination of reagents and equipment with the test strips RIDA®QUICK Gliadin (R7003/R7004)
- it is recommended to work **under a chemical hood,** because of ß-mercapto-ethanol in the Cocktail (patented) and the RIDA® Extraction Solution
- ß-mercaptoethanol can disturb the ELISA, therefore dilute the samples **at least 1:500** (recommendation 1:500 for samples with approx. 20 mg/kg gluten and 1:2500 for samples with approx. 100 mg/kg gluten).

9.2. Extraction with the Cocktail (patented) (R7006, official AOAC method)

Homogenize well a sufficient amount (at least 5 g or 5 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 and add 2.5 ml of the Cocktail (patented), close the vial and mix well
- **other food samples (e.g. soy and quinoa containing samples)**: weigh 0.25 g of the homogenized sample and add 2.5 ml of the 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 gliadin may be not distributed 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

Please further extract all samples as described in the following:

- incubate for 40 min at 50 °C (122 °F)
- let the sample cool down and then mix it with 7.5 ml 80 % ethanol (see 5.2.)
- close the vial and shake for 1 h up side 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) and/or filter the extract (alternatively 2 ml of the extract can be centrifuged with high speed for 10 min in reaction caps by using a microcentrifuge)
- put the supernatant in a screw top vial
- dilute the sample 1:12.5 (1+11.5 / 80 µl + 920 µl) with diluted sample diluent (see 10.1.): the final dilution factor is 500
- use **immediately** 100 µl per well in the assay

9.3. Extraction with the RIDA® Extraction Solution / Extraktions-Lösung (R7099)

The extraction with the RIDA® Extraction Solution / Extraktions-Lösung is more time saving than with Cocktail (patented).

Homogenize well a sufficient amount (at least 5 g or 5 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 and add 2.5 ml of the RIDA® Extraction Solution / Extraktions-Lösung, close the vial and mix well
- **other food samples**: weigh 0.25 g of the homogenized sample and add 2.5 ml of the RIDA® Extraction Solution / Extraktions-Lösung, close the vial and mix well
- **soy, millet, quinoa or buckwheat containing food samples as well as further tannin and polyphenol containing food samples (e.g. chocolate, coffee or cocoa, chestnut flour, spices)**: weigh 0.25 g of the homogenized sample, add 0.25 g of skimmed milk powder and add 2.5 ml of the RIDA® Extraction Solution / Extraktions-Lösung, close the vial and mix well
- **meat and sausages:** in these matrices gliadin may be not distributed evenly, therefore, weigh 50 g sample and homogenize
- weigh 0.25 g of the homogenized sample and add 2.5 ml of the RIDA® Extraction Solution / Extraktions-Lösung, close the vial and mix well

Please further extract all samples as described in the following:

- incubate for 15 min at 60 °C (122 °F)
- let the sample cool down and then mix it with 7.5 ml 68 % 2-propanol (see 5.2.)
- close the vial and incubate for 10 min in a water bath at 60 °C (140 °F)

- centrifuge: 10 min, at least 2500 g, at room temperature (20 - 25 °C / 68 - 77 °F) and/or filter the extract (alternatively 2 ml of the extract can be centrifuged with high speed for 10 min in reaction caps by using a microcentrifuge)
- put the supernatant in a screw top vial
- dilute the sample 1:12.5 (1+11.5 / 80 µl + 920 µl) with diluted sample diluent (see 10.1.): the final dilution factor is 500
- use **immediately** 100 µl per well in the assay

Remark:

The supernatant obtained after the centrifugation or the filtrate can be stored in a tightly closed vial in the dark at room temperature (20 - 25 °C / 68 - 77 °F) up to eight weeks.

10. Test implementation

10.1. Test preparation

Bring all reagents to room temperature (20 - 25 °C / 68 - 77 °F) before use.

The **sample diluent** is provided as a concentrate (5fold). Only the amount which actually is needed should be diluted 1:5 (1+4) with distilled water (e. g. 3 ml concentrate + 12 ml distilled water, sufficient for the dilution of 10 samples). Make sure that the buffer is not contaminated with gliadin.

The **antibody enzyme conjugate** (bottle with red cap) is provided as a concentrate (11fold). Since the diluted enzyme conjugate solution has a limited stability, only the amount which actually is needed should be diluted. Before pipetting, the conjugate concentrate should be shaken carefully. For reconstitution, the conjugate concentrate is diluted 1:11 (1+10) with distilled water (e. g. 200 µl concentrate + 2 ml distilled water, sufficient for 2 microtiter strips). Take care that the water is not contaminated with gliadin.

The **washing buffer** is provided as a 10fold concentrate. Before use, the buffer has to be diluted 1:10 (1+9) with distilled water (i.e. 100 ml buffer concentrate + 900 ml dist. water). Prior to dilution, dissolve eventually formed crystals by incubating the buffer in a water bath at 37 °C (99 °F). The diluted buffer is stable at 2 - 8 °C (35 - 46 °F) for four weeks.

10.2. Test procedure

Carefully follow the recommended washing procedure. Do not allow microwells to dry between working steps.

1. Insert a sufficient number of wells into the microwell holder for all standards and samples to be run in duplicate. Record standard and sample positions.
2. Add 100 µl of each standard solution or sample to separate duplicate wells and incubate for 30 min at room temperature (20 - 25 °C / 68 - 77 °F).
3. Pour the liquid out of the wells and tap the microwell holder upside down vigorously (three times) against absorbent paper to ensure complete removal of liquid from the wells. Fill all the wells with 250 µl diluted washing buffer (see 10.1.) and pour out the liquid again. Repeat two more times.
4. Add 100 µl of the diluted enzyme conjugate (see 10.1.) to each well and incubate for 30 min at room temperature (20 - 25 °C / 68 - 77 °F).
5. Pour the liquid out of the wells and tap the microwell holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. Fill all the wells with 250 µl diluted washing buffer (see 10.1.) and pour out the liquid again. Repeat two more times.
6. Add 50 µl of substrate and 50 µl of chromogen to each well. Mix gently by skaking the plate manually and incubate for 30 min at room temperature (20 - 25 °C / 68 - 77 °F) in the dark.
7. Add 100 µl of the stop reagent to each well. Mix gently by skaking the plate manually and measure the absorbance at 450 nm. Read within 30 min after addition of stop solution.

11. Results

A special software, the RIDA®SOFT Win (Z9999), is available for evaluation of the RIDASCREEN® enzyme immunoassays. The calculation should be done by use of a cubic spline function.

The course of the standard curve is shown in the Quality Assurance Certificate enclosed in the test kit. For quality assurance Gliadin Assay Controls (R7010) should be used.

In comparison with the certificate, higher values of the absorbance ($A_{450\ nm}$) for the calibration curve, especially for the zero standard, may be a result of insufficient washing or gliadin contamination.

A further dilution and new detection of the samples is recommended for absorbance values ($A_{450\ nm}$) > standard 6. The samples should be diluted so that the results can be read in the linear part of the calibration curve.

The gliadin concentration in µg/kg (ppb) is read from the RIDA®SOFT Win calibration curve and must be further multiplied by the recommended dilution factor of 500. This result is then multiplied by 2 in order to obtain the gluten concentration (gliadin usually represents 50 % of the proteins present in gluten).

Example:
The absorbance value of a sample corresponds to 10 µg/kg gliadin in the calibration curve. Multiplying by the recommended dilution factor 500 leads to 5000 µg/kg, corresponding to 5 mg/kg (ppm) gliadin, respectively 0.0005 % gliadin. To calculate the gluten content, it is necessary to multiply by factor 2 which results in 10 ppm gluten, respectively 0.001 % gluten. This sample is considered to be gluten-free, because the gluten concentration is below 20 ppm.

The product information folder with further information is available on request from your local distributor or R-Biopharm AG.