

# RIDASCREEN<sup>®</sup> Total Gluten

Art. No. R7041

## Analysis of oat-free food samples or samples with low oat content with RIDASCREEN<sup>®</sup> Total Gluten test

### Information

The application note describes the analysis of **oat-free** food samples and food samples with **low oat content** (< 50 %) in the RIDASCREEN<sup>®</sup> Total Gluten ELISA (Art. No. R7041). It is recommended to analyze samples with **unknown oat** content according to this application note too.

The RIDASCREEN<sup>®</sup> Total Gluten ELISA was developed and validated for oat matrices. In the course of further development of the ELISA, the use of RIDASCREEN<sup>®</sup> Total Gluten was also validated for oat-free food samples and samples with low oat content (< 50 %). An additional reagent, the **RIDASCREEN<sup>®</sup> Total Gluten Additive TG** (Art. No. RA0041), is required for this analysis.

In this application note, the following non-oat food samples were validated for analysis in RIDASCREEN<sup>®</sup> Total Gluten: rice flour, corn flour, soy flour, cookies, sausage, starch, pseudo cereals, legumes and vegetarian meat alternatives. It can be assumed that the test with the addition of Additive TG is also suitable for the analysis of other foods; this is to be verified by the user himself.

### General

Sample extracts must be diluted prior to use in the ELISA due to the cocktail used for extraction. However, food samples with an oat content < 50 % show matrix-specific differences in the RIDASCREEN<sup>®</sup> Total Gluten ELISA compared to samples with a high oat content or to pure oat samples. To compensate this, the additive reagent Additive TG was developed for the dilution of samples extracts with an oat content < 50 %. Then, the diluted sample extracts are analyzed according to the test procedure in chapter 10. of the instructions for use of the RIDASCREEN<sup>®</sup> Total Gluten test kit.

In case of contamination, pure oat samples (oat grains, oat flakes, etc.) often show an inhomogeneous distribution of gluten. For this reason, it is recommended to homogenize at least 200 g of such samples and to use 1 g of this for the extraction. However, for food samples with a low oat content, it is usually sufficient to take a smaller amount of the homogenized sample (0.25 g) for extraction. This allows a more economical use of the reagents. This application note therefore describes the extraction of smaller sample quantities than in the RIDASCREEN<sup>®</sup> Total Gluten instructions for use.

### Reagent provided within RIDASCREEN<sup>®</sup> Total Gluten Additive TG (Art. No. RA0041)

1 x 2 mL Additive TG (sufficient for 100 samples)

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### Reagents required but not provided

For additionally required reagents refer to RIDASCREEN® Total Gluten (Art. No. R7041) instructions for use, see chapter 5.2.

### Storage of RIDASCREEN® Total Gluten Additive TG (Art. No. RA0041)

Store the Additive TG at 2 - 8 °C (35 - 46 °F). Please do not freeze.

Do not use the Additive TG after the expiration date (see test kit label).

Bring the Additive TG to room temperature (20 - 25 °C/68 - 77 °F) before use.

For the use of the Additive TG, the precautions of the implementation instructions of the RIDASCREEN® Total Gluten apply.

### Sample preparation

The specifications of the RIDASCREEN® Total Gluten (Art. No. R7041) instructions for use apply. Deviating from this, the sample homogenization can be performed as follows to allow a more economical use of the reagents:

Homogenize (grind thoroughly to powder and mix well or mix well a solution respectively) well a sufficient amount (e.g. 50 g or 50 mL) to ensure taking a representative test portion of sample.

- Weigh in  $0.25 \pm 0.01$  g of homogenized sample to a new centrifugal vial.  
In case of samples containing tannin and polyphenols (see also chapter 9.2. of the RIDASCREEN® Total Gluten instructions for use), add 0.25 g skim milk powder to the sample.
- Add 2.5 mL of Cocktail (patented), close the vial and mix well (e.g. vortexer).
- Pay attention to obtain a homogeneous suspension!
- Add 7.5 mL 80 % ethanol (see chapter 5.), close the vial and mix well (e.g. vortexer).
- Pay attention to obtain a homogeneous suspension!

**Note:** The weighted sample can be further increased for very inhomogeneous samples (> 0.25 g). In this case, the volume of Cocktail (patented) and 80 % ethanol must be increased accordingly. If used, skim milk powder amount needs to be increased too.

Then, the sample is extracted in a water bath and further processed according to the instructions for use of the RIDASCREEN® Total Gluten test.

The sample extracts (supernatant from centrifugation step or filtrate) can be stored undiluted in a well-sealed container at room temperature (20 - 25 °C/68 - 77 °F) (shelf life approx. 2 weeks) until used in the test.

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## Use of Additive TG (test procedure)

The  $\beta$ -mercaptoethanol contained in the Cocktail (patented) may interfere in the ELISA. Therefore, the extracts (supernatant of the centrifugation step or the filtrate) must always be further diluted before use in the test. In the case of oat-free samples, samples with a low oat content as well as samples with unknown oat content, dilution with **buffer with Additive TG** is performed to compensate for the matrix-specific differences to samples containing oats.

## Preparation of buffer with Additive TG

For use in the test, the Additive TG must be mixed 1:50 with the buffer contained in the RIDASCREEN® Total Gluten test kit (Art. No. R7041).

### Example:

Amount of samples	Buffer	Additive TG
1	980 $\mu$ L	20 $\mu$ L
10	9800 $\mu$ L	200 $\mu$ L

The **buffer with Additive TG** should be prepared immediately before dilution of the sample extracts (use within 30 minutes).

- Dilute extracts 1:25 with **buffer with Additive TG** (e.g. 960  $\mu$ L buffer with Additive TG + 40  $\mu$ L extract). The final dilution factor is 1:1000.
- Use the diluted sample extracts immediately (within 30 minutes) in the assay. A longer period may influence the recovery.
- Follow the test procedure according to RIDASCREEN® Total Gluten test kit (Art. No. R7041) instructions for use, see chapter 10.

If samples have a higher absorbance than standard 6, sample extracts can be further diluted and retested in the ELISA. For this, dilute the extract again 1:25 with buffer with Additive TG as described. Then perform the additional dilution of the diluted sample extract with the following buffer:

- 1 % Cocktail (patented)
- 3 % of an 80 % ethanol solution
- 96 % buffer without Additive TG

e.g. 50  $\mu$ L Cocktail (patented), 150  $\mu$ L 80 % ethanol solution and 4800  $\mu$ L buffer without Additive TG.

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## Specifications/results

LoD (Limit of Detection)	2.8 mg/kg gluten (1.77 - 3.84 mg/kg)
LoQ (Limit of Quantification)	5 mg/kg gluten
Measuring range	5 - 80 mg/kg gluten
Dilution factor	1,000
Robustness	When performing the test, the specified temperature must be complied. Deviations from this can influence the recovery.

The specifications were determined with spiked and incurred samples. Naturally contaminated samples may lead to deviating results.

## Disclaimer

The information in this application note is given as a hint for analysis of oat-free food samples, samples with low oat content (< 50 %) and samples with unknown oat content in RIDASCREEN® Total Gluten test kit (Art. No. R7041) using the RIDASCREEN® Total Gluten Additive TG (Art. No. RA0041) only and shall not be regarded as any description or warranty of a certain condition, quality, or functionality of RIDASCREEN® Total Gluten.

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