Test Instruction

Gliadin/Gluten

96/48 Tests

Enzyme Immunoassay
for the Quantitative
Determination of
Gliadin/Gluten in Food

Cat.-No.: GLU-E02/E04

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This document represents a combined test instruction for the products GLU-E02 (96 well) and GLU-E04 (48 well).

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Sensitivity (Gliadin), beer	0.03 ppm
Sensitivtty (Gliadin), others	0.3 ppm
Recovery	85-98%
Incubation Time	60 min

General Information

Gluten is the main part of the protein fraction of cereals and consists of nearly the equal amount of the protein compounds prolamin (gliadin) and glutenin. Because of its special physico-chemical attributes and its low price, gluten is not only contained in cereal products, but also in other food as sausage products and ice cream or in drugs and cosmetics as binder and filler.
For some persons, gluten has a pathological effect (coeliac disease). These people need to have a strict gluten free diet. In the European Union a maximum level of 20 ppm gluten is allowed for products declared as “gluten-free”, and 100 ppm gluten for products declared as “very low gluten” respectively. Sensitive detection systems are required to determine gluten residues in foodstuff.
The **Eurofins Immunolab Gliadin/Gluten ELISA** represents a highly sensitive detection system and is particularly capable of the quantification of gliadin/gluten residues in bakery products, baby food, beer, meat and chocolate.

Principle of the Test

The **Eurofins Immunolab Gliadin/Gluten** quantitative test is based on the principle of the enzyme-linked immunosorbent assay. An antibody directed against gliadin – the soluble fraction of gluten - is bound on the surface of a microtiter plate. Gliadin containing samples or standards are given into the wells of the microtiter plate. After 20 minutes of incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugated second antibody directed against gliadin is given into the wells and after 20 minutes of incubation, the plate is washed again. A substrate solution is added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of gliadin is directly proportional to the colour intensity of the test sample. Because of the equal amounts of gliadin and glutenin in wheat gluten, the gluten concentration of the sample is calculated by multiplication with the factor 2.

Precautions

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipettes, ELISA reader etc.).

Health and safety instructions

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

Reagents

The kit contains reagents for 96/48 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1. Microtiter plate consisting of 12/6 strips with 8 breakable wells each, coated with anti-gliadin.
2. Gliadin Standards (0, 2, 6, 20, 60 ppm Gliadin): 1 x 5 vials with 2.0 mL each, dyed red, ready-to-use.
3. Conjugate (anti-gliadin-Peroxidase): 15/7.5 mL, dyed red, ready-to-use.
4. Substrate Solution (TMB): 15 mL, ready-to-use.
5. Stop Solution (0.5 M H_2SO_4): 15 mL, ready-to-use.
6. Sample dilution buffer (Tris): 60 mL as 10x concentrate, dyed red. Dilute 1+9 with distilled water. Stored at 4°C the diluted buffer is stable for at least one week. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
7. Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
8. Two plastic foils to cover the strips during the incubation.
9. Plastic bag to store unused microtiter strips.
10. Instruction Manual.

Additional Instrumentation and Reagents (not provided)

Instrumentation

- 5 - 1000 µL-micropipets
- ELISA reader (450 nm)
- Ultra-Turrax or mixer
- Centrifuge

Reagents

- Ethanol (40%)
- distilled water
- Skimmed milk powder when indicated

Sample Preparation

Due to the high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be **cleaned thoroughly** before and after each sample. To identify possible cross-contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions.

The following sample preparation should be applied for beer samples:

1. Degas beer, for instance by ultrasonics.

2. Dilute 100 µL of beer with 4.9 mL of **pre-diluted** sample dilution buffer.

3. The samples are centrifuged for 10 minutes at 2500 g. The particle-free supernatant has to be applied in the test procedure.

The following sample preparation should be applied for all other kinds of samples:

1. To maximize homogeneity and representativeness of the sample collection, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill etc.

2. 1 g of the homogenized mixture is suspended in 10 mL of 40% ethanol. If tannin-containing food like chocolate is extracted, 1 g of skimmed milk powder is added before suspension. Afterwards the suspension is mixed for further 5 min to ensure good homogeneity.

3. The samples are centrifuged for 10 minutes at 2500 g. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.

4. Afterwards the particle-free solution is diluted 1:50 in **pre-diluted** sample dilution buffer (for example 20 µL of solution in 980 µL sample dilution buffer).

If the results of a sample are out of the measuring range, further dilution with the **pre-diluted** sample dilution buffer is necessary. The additional dilution has to be considered when calculating the concentration.

Procedure

The washing solution is supplied as 10x concentrate and has to be **diluted** 1+9 with double distilled water before use.

In any case the **ready-to-use** standards provided should be determined twofold. When samples in great quantities are determined, the standards should be pipetted once before the samples and once after the samples. For final interpretation the arithmetic mean is used for calculation.

In consideration of GLP and quality management a duplicate measurement of samples is recommended.

The procedure is according to the following scheme:

1. Prepare samples as described above.

2. Pipet 100 µL **ready-to-use** standards or prepared samples in duplicate into the appropriate wells of the microtiter plate.

3. Incubate for 20 minutes at room temperature.

4. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.
5. Pipet 100 µL of conjugate (anti-gliadin-peroxidase) into each well.

6. Incubate for 20 minutes at room temperature.

7. Wash the plate as outlined in 4.

8. Pipet 100 µL of substrate solution into each well.

9. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 20 minutes at room temperature.

10. Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H_2SO_4) into each well. The blue colour will turn yellow upon addition.

11. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

Calculation of results

The ready-to-use standards are prepared for a direct determination of sample concentrations. **The dilution of samples in the extraction process as described in the above stated sample preparation procedure is already considered. In case of beer samples, the resulting concentration has to be divided by 10.** Additional dilution due to high sample concentration has to be accounted for.

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.

2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ppm on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis. Alternatively, the evaluation can

be carried out by software. In this case the 4-parameter method should be preferred.

3. Using the mean optical density value for each sample, determine the corresponding concentration of gliadin in ppm from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

4. For calculating the corresponding gluten concentration, the result of gliadin has to be multiplicated with factor 2.

Typical Standard Values

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 60 ppm standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

Gliadin (ppm)	% binding of 60 ppm
60	100
20	62
6	27
2	13
0	6

Performance

Sensitivity

The limit of detection (LOD) of the **Eurofins Immunolab Gliadin/Gluten test** is 0.03 ppm Gliadin for beer samples and 0.3 ppm Gliadin for all other samples.

The limit of quantification (LOQ) of the **Eurofins Immunolab Gliadin/Gluten test** is 0.2 ppm Gliadin for beer samples and 2 ppm Gliadin for all other samples.

Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

Cross-reactivity

For the following foods no cross-reactivity could be detected:

Amaranth	Egg	Quinoa
Beef	Milk	Rice
Buckwheat	Millet	Soy
Cocoa	Teff	Cocoa
Corn		

The gluten concentration in various foods may vary significantly. Additionally, the determined concentration of different cereals depends on the cross-reactivity of the prolamin towards the wheat-gliadin antibody. The following table will give guidelines for the cross-reactivity of different cereals.

Barley	14%
Oats	0.06%
Rye	63%
Spelt	13%
Triticale	56%
Wheat	100%

Precision

Intra-assay Precision	4 - 5%
Inter-assay Precision	2 - 3%

Linearity

The serial dilution of spiked samples (rice wafer, corn semolina, dark chocolate, sausage, baby food, and beer) resulted in a dilution linearity of 95% - 115%.

Recovery

Mean recovery was determined by spiking samples with different amounts of gliadin:

Rice wafer	97%
Corn semolina	98%
Dark chocolate	97%
Baby food	88%
Sausage	85%
Beer	96%

References

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3. Collin P, et al. (2004) – The safe threshold for gluten contamination in gluten-free products. Can trace amounts be accepted in the treatment of celiac disease?. Aliment Pharmac Ther 19:1277-1283
4. Hernando A, et al. (2008) – Measurement of wheat gluten and barley hordeins in contaminated oats from Europe, the United States and Canada by sandwich R5 ELISA. Eur J Gastr Hepat, 20(6):545-54
5. Thompson T, et al. (2008) – Commercial assays to assess gluten content of gluten-free foods: why they are not created equally. J Am Diet Assoc, 108(10):1682-7