**GLUTEN**

| COD 31000<br>1 x 20 mL + 1 x 5 mL | COD 31001<br>2 x 20 mL + 2 x 5 mL |
|---|---|
| Only for *in vitro* use in the laboratory | |

**GLUTEN**
IMMUNOTURBIDIMETRY

## INTENDED USE

Reagent for the detection and quantification of gluten through the immunotoxic fraction of the prolamins from wheat (gliadin), rye (secalin) and barley (hordein) in raw products such as flours (buckwheat, rice, corn, oats) and spices, as well as in processed foods such as ready-to-eat meals, dairy products, chocolate, bakery products, processed meats, wine and other beverages (Note 1,2).

Use BioSystems Gluten Extraction Solution to extract gluten from the samples.

The measurement range of the kit is from 2.5 to 40 mg/kg of gluten. Samples with a concentration above the specified linearity limit can be diluted accordingly with BioSystems Gluten Extraction Solution.

## PRINCIPLE OF THE METHOD

Gliadin in the sample causes agglutination of latex particles coated with a monoclonal antibody specific for the 33-mer sequence. The agglutination of the latex particles is proportional to the concentration of gliadin and can be quantified by turbidimetry.

## CONTENTS

| | COD 31000 | COD 31001 |
|---|---|---|
| A. Reagent | 1 x 20 mL | 2 x 20 mL |
| B. Reagent | 1 x 5 mL | 2 x 5 mL |
| S. Standard | 5 x 5 mL | 5 x 5 mL |

## COMPOSITION

A. Reagent. Buffer, sodium azide 0.95 g/L.

B. Reagent. Suspension of latex particles coated with anti-Gliadin 33-mer monoclonal antibody, sodium azide 0.95 g/L.

S1. Standard. 1 x 5 mL. Gliadin PWG 0.031 mg/L. Aqueous primary standard.

S2. Standard. 1 x 5 mL. Gliadin PWG 0.063 mg/L. Aqueous primary standard.

S3. Standard. 1 x 5 mL. Gliadin PWG 0.125 mg/L. Aqueous primary standard.

S4. Standard. 1 x 5 mL. Gliadin PWG 0.250 mg/L. Aqueous primary standard.

S5. Standard. 1 x 5 mL. Gliadin PWG 0.500 mg/L. Aqueous primary standard. (Note 3)

*WARNING: H226: Flammable liquid and vapour. H319: Causes serious eye irritation. P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P403+P235: Store in a well-ventilated place. Keep cool.*

For further warnings and precautions, see the product safety data sheet (SDS).

## STORAGE AND STABILITY

Store at 2-8ºC.

Components are stable once opened until the expiry date marked in the label if they are stored well closed and care is taken to prevent contamination during their use.

Indications of deterioration: Absorbance of the blank over 0.800A.

## WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines. Any serious incident that might occur in relation to the device shall be reported to BioSystems S.A.

## ADDITIONAL EQUIPMENT AND REAGENTS

BioSystems analyzer, spectrophotometer or photometer with cell holder thermostatable at 37ºC and able to read at 520 nm.

Analytical balance.

Microcentrifuge vials and/or tubes for centrifugation.

Homogenizer: Laboratory mincer/grinder and vortex.

Centrifuge.

Incubator or water bath (50 ºC).

Syringe filters (e.g., Whatman Cat. No. 6884-2510 or similar).

BioSystems Gluten Extraction Solution (Ref. 31003).

BioSystems Gluten Spike Solution (Ref. 31002). (Note 4)

## REAGENTS PREPARATION

All reagents and standards are provided ready to use.

## PROCEDURE

**Sample preparation**

Solids:

1. Treat, prepare and homogenize the sample according to a protocol that guarantees a representative sample without alterations in the natural content of gluten. It is recommended to thoroughly grind and homogenize a representative amount of the sample (e.g., 200 g) before extracting the test portion.
2. Weigh 0.25 g (±0.01 g) of homogenized solid sample into a screw-cap vial and add 10 mL of BioSystems Gluten Extraction Solution (Ref. 31003).
3. In cases where the distribution of gluten within the sample is highly heterogeneous and/or challenging to homogenize (e.g. oat sample), increase the weight of the homogenized sample and, consequently, the volume of BioSystems Gluten Extraction Solution. For example, consider using a 0.5 g sample with 20 mL of BioSystems Gluten Extraction Solution or a 1 g sample with 40 mL of BioSystems Gluten Extraction Solution.
4. No special treatment is necessary for food samples containing tannins and/or polyphenols (e.g., chocolate, coffee, cocoa, chestnut flour, buckwheat, millet, spices…).
5. Close the vial and shake using a vortex mixer or similar for 30 seconds, until the sample is homogeneously suspended .
6. Incubate the mix for 40 minutes at 50 ºC in a water bath.
7. Remove samples and let cool to room temperature (5 - 10 minutes).
8. Centrifuge for 10 minutes, at least at 2000 g. or transfer a portion of suspension (e.g., 1.5 mL) to a microcentrifuge vial and centrifuge at high speed (≥10000 g) for 2 minutes.
9. If a layer of fat is observed after centrifugation, take the supernatant through it, pipette into another vial and centrifuge again. If the supernatant is still turbid, filter with a syringe filter (e.g. Whatman Cat. No. 6884-2510 or similar).
10. Gluten in the supernatant is stable for at least 8 days at 15-25 ºC.

Liquids:

1. Treat, prepare and homogenize the sample according to a protocol that guarantees a representative sample without alterations in the natural content of gluten.
2. Pippete 0.25 mL of homogenized sample into a screw-cap vial and add 10 mL of BioSystems Gluten Extraction Solution.
3. In cases where the distribution of gluten within the sample is highly heterogeneous and/or challenging to homogenize, increase the volume of the homogenized sample and, consequently, the volume of BioSystems Gluten Extraction Solution. For example, consider using a 0.5 mL sample with 20 mL of BioSystems Gluten Extraction Solution or a 1 mL sample with 40 mL of BioSystems Gluten Extraction Solution.
4. No special treatment is necessary for food samples containing tannins and/or polyphenols (e.g., wine).
5. Close the vial, shake and incubate the mixture for 10 minutes at room temperature.
6. Use the sample directly for gluten determination.
7. Gluten in the sample is stable for at least 8 days at 15-25 ºC.

**Manual procedure**

1. Bring the reagents and the instrument to reaction temperature (37ºC).
2. Pipette into a cuvette (Note 5-9):

| | Reagent Blank (RB) | Standards/Sample |
|---|---|---|
| Standards/Sample | - | 100 µL |
| BioS GlutExtract Sol. | 100 µL | - |
| Reagent A | 800 µL | 800 µL |

3. Mix and incubate for 1 minute at 37ºC. Read absorbance (A1) at 520 nm.
4. Pipette into the cuvette:

| | | |
|---|---|---|
| Reagent B | 200 µL | 200 µL |

5. Mix and incubate for 10 minutes at 37 ºC. Read absorbance (A2) at 520 nm.
6. Calculate increase of absorbance of standard/sample using the following formula:

$$A = (A2 - 0.82 \times A1)_{Standard/Sample} - (A2 - 0.82 \times A1)_{RB}$$

7. Calculate the gliadin concentration (C) using the calibration curve, then convert it to gluten concentration by multiplying the gliadin concentration by 2 (Note 3).
8. Samples with concentration over the specified linearity limit should be accordingly diluted with BioSystems Gluten Extraction Solution. Multiply obtained concentration by the dilution factor (df).
9. When analyzing solid and semi-solid samples which are weighed out for sample preparation, the content (mg/kg) (CT) is calculated from the amount of sample weighed (W), the volume in which weighed sample is prepared (V), the gliadin concentration obtained in the sample (C) and the dilution factor (df) if necessary, as follows:

$$\frac{C_{Sample}\,(mg/L) \times V\,(L)}{W_{Sample}\,(kg)} \times df = CT_{Sample}\,[mg/kg]$$

**Automated procedure**

1. Transfer at least 500 µL the supernatant to a vial or tube for the BioSystems Y15 analyzer.
2. Arrange the samples, calibrators, and reagents on the analyzer according to BioSystems Y15 instructions (Note 5, 7, 8, 9).
3. Program the session on the BioSystems Y15 analyzer following the guidelines provided in the user manual.

4. Use the GLIADIN test for analysis. The parameters for this test are programmed in the analyzer as shown below:

| | |
|---|---|
| GENERAL | |
| Test name | **GLIADIN** |
| Analysis mode | Differential bireagent |
| Sample type | ST1 |
| Units | mg/L |
| Turbidimetry test | Yes |
| Active mixing | Yes |
| Reaction type | Increasing |
| Replicates | 2 |
| Decimals | 3 |
| PROCEDURE | |
| Reading | Monochromatic |
| Sample | 20 |
| Reagent 1 | 160 |
| Reagent 2 | 40 |
| Washing | 1.2 |
| Predilution factor | - |
| Main filter | 520 |
| Reference filter | - |
| Reading 1 | 96 s |
| Reading 2 | 600 s |
| Reagent 2 | 120 s |
| CALIBRATION | |
| Calibration type | Specific |
| Calibration curve | 5 |
| OPTIONS | |
| Solution for blank | Specific [Diluent 1] (Note 9) |
| Blank absorbance limit | 0.800 |
| Kinetic blank limit | - |
| Linearity limit | 0.5 |

5. To obtain the concentration of gluten in mg/L, use the Calculated Test "GLUTEN (mg/L)". To obtain the concentration of gluten in mg/kg, use the Calculated Test "GLUTEN (mg/kg)". Enter the exact weight (g) for each sample. All calculations are done automatically by the analyzer software (Note 3).
6. Samples with concentration over the specified linearity limit should be accordingly diluted with BioSystems Gluten Extraction Solution.

## CALIBRATION

A reagent blank (Note 9) should be done every day and a calibration after reagent lot change or as required by quality control procedures.

## QUALITY CONTROL

To ensure an accurate and precise result, free from matrix effects, it is recommended to test samples with the addition of a known concentration of gluten as test controls. For this purpose, BioSystems Gluten Spike Solution should be used. (Note 4)

Each laboratory should establish its own internal quality control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

## PERFORMANCE CHARACTERISTICS

The metrological characteristics described below have been obtained using a Y15 analyzer. Details on evaluation data are available on request.

- Limit of quantification: 2.5 mg/kg (mg/L) gluten
- Measurement interval: 2.5 - 40 mg/kg (mg/L) gluten
- Precision: An example of matrices, sources of contamination, and levels of gluten concentration is shown. More data from the precision study is available on request.

- Precision:

| Matrices | Gluten contamination | | RSDr |
|---|---|---|---|
| | Source | mg/kg | |
| Corn flour | Wheat flour | 5 | 10.7 |
| | | 20 | 8.4 |
| Rice flour | Wheat flour | 5 | 9.2 |
| | | 20 | 11.7 |
| Red wine | Wheat flour | 5 | 3.5 |
| | | 10 | 3.4 |
| Sausage | Wheat flour | 5 | 8.0 |
| | | 20 | 10.5 |
| Cacao powder | Gluten Spike Solution | 5 | 7.1 |
| | | 10 | 3.6 |
| Cookies | Gluten Spike Solution | 2.5 | 4.1 |
| | | 10 | 2.0 |

- Trueness: Results obtained with this procedure did not show systematic differences when compared with a reference procedure. Details of the comparison experiments are available on request.
- Specificity: This method employs an antibody directed against the immunotoxic 33-mer fraction of prolamins and is specific to wheat (gliadin), rye (secalin), and barley (hordein). No cross-reactivity has been detected with other gluten-free foods (Note 4).
- Prozone effect: No prozone effect was observed within the studied range (0 - 85000 mg/kg gluten).

## NOTES

1. This method has not been validated for fermented or hydrolyzed foods (e.g. beer or sourdough).
2. In cases where the presence of gluten in wine is associated with the use of plant-based fining agents, enological practices such as sealing barrels with wheat paste, or potential contaminations following fermentetion.
3. Standard concentrations are given in gliadin. They have been prepared from the reference material PWG-Gliadin (Prolamin Working Group). To convert the gliadin concentration to gluten, a conversion factor of 2 is used, as defined by the Codex Alimentarius.
4. The assessment of cross-reactivity was performed by analyzing a single representative sample from each individual matrix type. The results obtained from these samples are presented in the performance evaluation report. It is important to note that alternative samples may yield different results, hence it is recommended to carry out recovery studies.
5. Shake the Reagent B vial gently before using.
6. Volumes proposed are to use a semi-micro cuvette. Other volumes can be used if the ratio between the reagents and sample is maintained
7. Do not interchange individual reagents between kits of different lot numbers.
8. It is recommended to measure the samples in duplicate.
9. Perform the reagent blank using BioSystems Gluten Extraction Solution (Ref. 31003).

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