GLUTEN

COD 31000 1 x 20 mL + 1 x 5 mL	COD 31001 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 200 mg/kg of gluten. Samples with a concentration above the specified linearity limit can be diluted accordingly with BioSystems Gluten Extraction Solution.

The scope of AOAC Performance Tested Methods℠ certification (PTM #072503) includes rice flour, corn flour, sausage, rice cookies, cornbread and post-fermentation wine (Note 2) samples for automated procedure with BioSystems analyzers[1].

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 2-8 ºC.

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 1.0 Abs.

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional users 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 37 ºC and able to read at 520 nm.

Analytical balance.

Capped tubes for centrifugation.

Homogenizer: laboratory mincer/grinder and vortex.

Centrifuge.

Incubator or water bath (50 ºC).

BioSystems Gluten Extraction Solution (Cod. 31003).

BioSystems Gluten Spike Solution (Cod. 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 tube and add 10 mL of BioSystems Gluten Extraction Solution (Cod. 31003).
3. In the case of oat samples where the distribution of gluten within the sample is highly heterogeneous or in cases where the sample is challenging to homogenize, increase the weight of the homogenized sample and, consequently, the volume of BioSystems Gluten Extraction Solution. Use 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.
9. 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 tube and add 10 mL of BioSystems Gluten Extraction Solution.
3. In cases where the sample is challenging to homogenize, increase the volume of the homogenized sample and, consequently, the volume of BioSystems Gluten Extraction Solution. Use 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 (37 ºC).
2. Pipette into a cuvette (Note 5, 8):

	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 37 ºC. 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).
3. Program the session on the BioSystems Y15 analyzer following the guidelines provided in the user manual.

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

GLIADIN	ST1 / FOOD / WINE	ST2 / FOOD2 / WINE2
GENERAL PARAMETERS		
UNITS	mg/L	mg/L
DECIMALS	4	4
REPLICATES	2	2
REACTION TIME	Increasing	Increasing
TURBIDIMETRY TEST OPTION	Yes	Yes
ACTIVE MIXING OPTION	Yes	Yes
PROCEDURE PARAMETERS		
READING MODE	Monochromatic	Monochromatic
MAIN FILTER	520	520
REFERENCE FILTER	-	-
SAMPLE VOLUME	20	20
REAGENT A VOLUME	160	160
REAGENT B VOLUME	40	40
READING TIME 1 CYCLE (S)	5 (96)	5 (96)
READING TIME 2 CYCLE (S)	26 (600)	26 (600)
REAGENT B TIME CYCLE (S)	6 (120)	6 (120)
DILUTION PARAMETERS		
DILUTION ACTIVE	-	Yes
MODE	-	By Analyser
DILUTION FACTOR 1/	-	5
DILUENT	-	Gluten extraction solution
CALIBRATION PARAMETERS		
CALIBRATION MODE	Experimental	Alternative
ALTERNATIVE SAMPLE-TYPE	-	WINE
SOLUTION FOR BLANK	Gluten extraction solution	-
BLANK REPLICATES	3	-
CALIBRATION FACTOR	-	-
CALIBRATION REPLICATES	3	-
NAME	Gluten Standard	-
NUMBER CALIBRATION POINTS	5	-
DILUTION MODE	By User	-
CURVE	Increasing	-
CALIBRATION FUNCTION	4 parameter logistic (4PL)	-
CONCENTRATION (1)	0.031	-
CONCENTRATION (2)	0.063	-
CONCENTRATION (3)	0.125	-
CONCENTRATION (4)	0.25	-
CONCENTRATION (5)	0.5	-
LIMIT PARAMETERS		
BLANK ABSORBANCES LIMIT	1.0	1.0
LINEARITY LIMIT	0.5	2.5

5. To obtain the gluten concentration, use the following Calculated Tests programmed in the analyzer's software:

Calculated Test	Unit	Sample	Sample Type	Interval
GLUTEN 40 (mg/kg)	mg/kg	Solid	ST1/FOOD	2.5 - 40
GLUTEN 200 (mg/kg)	mg/kg	Solid	ST2/FOOD2	12.5 - 200
GLUTEN 40 (mg/L)	mg/L	Liquid	ST1/WINE	2.5 - 40
GLUTEN 200 (mg/L)	mg/L	Liquid	ST2/WINE2	12.5 - 200

6. The analyzer is programmed by default with a sample weight of 0.25 g (solid samples) or a volume of 0.25 mL (liquid samples). The user can modify these settings with the exact weight or volume of each sample.
7. All calculations are done automatically by the analyzer software (Note 3).
8. Samples with concentration over the specified linearity limit should be accordingly diluted with BioSystems Gluten Extraction Solution.

CALIBRATION

A reagent blank 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: ST1/FOOD/WINE: 2.5 mg/kg (mg/L) gluten.
  ST2/FOOD2/WINE2: 12.5 mg/kg (mg/L) gluten.
- Measurement interval: ST1/FOOD/WINE: 2.5 - 40 mg/kg (mg/L) gluten.
  ST2/FOOD2/WINE2: 12.5 -200 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.

Matrices	Gluten contamination		Recovery	RSDr
	Source	mg/kg	%	%
Corn flour	Wheat flour	5	98	16.1
		20	98	10.3
Rice flour	Wheat flour	5	120	5.91
		20	104	10.7
Red wine (post-fermentation)	Wheat flour	5	100	3.15
		10	96	4.08
Sausage	Wheat flour	5	153	9.38
		20	102	8.54
Instant Cacao powder	Gluten Spike Solution	5	87	7.1
		10	87	3.6
Cookies	Gluten Spike Solution	2.5	94	4.1
		10	99	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 fermentation[2].
3. Standard concentrations are expressed as gliadin and are traceable to the PWG-Gliadin reference material (Prolamin Working Group). To convert gliadin concentrations to gluten, a conversion factor of 2 is applied, in accordance with Codex Alimentarius guidelines[3].
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.

BIBLIOGRAPHY

1. AOAC Gluten Working Group, “Guidelines for Validation of Quantitative Gluten Methods, with Specific Examples for ELISA Assays,” Rockville, Maryland 20850, Jun. 2025.
2. International Organisation of Vine and Wine (OIV) Practical guide for the validation, quality control, and uncertainty assessment of an alternative oenological analysis method.
3. Codex Alimentarius Commission. Codex standard 118-1979. Foods for special dietary use for persons intolerant to gluten, in Codex Alimentarium. rev 2008: FAO: Rome, Italy; WHO: Geneva, Switzerland.

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