CERTIFICATION

AOAC Research Institute
Performance Tested Methods℠

Certificate No.

072503

The AOAC Research Institute hereby certifies the method known as

BioSystems Gluten Immunoturbidimetry

manufactured by

BioSystems S.A.
Costa Brava 30
08030 Barcelona, Spain

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*℠ Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*℠ certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Bradley A. Stawick, AOAC Research Institute Senior Director

Issue Date July 30, 2025

Expiration Date December 31, 2025

METHOD NAME	CATALOG NUMBERS	ORIGINAL CERTIFICATION DATE
BioSystems Gluten Immunoturbidimetry	31000, 31001	July 30, 2025

PRINCIPLE OF THE METHOD

The BioSystems Gluten method is a turbidimetric immunoassay designed for the quantification of gluten content derived from wheat, barley, and rye in food and beverage samples. The assay is performed on the BioSystems Y15 automated analyzer, which enables fully automated sample handling, reagent dispensing, and measurement.

The method utilizes a monoclonal antibody specifically developed to recognize the 33-mer peptide fragment of gliadin, a sequence characterized by high immunogenicity and toxicity in individuals with celiac disease. This antibody is immobilized and stabilized on the surface of polystyrene nanoparticles, forming the basis of the immunoreagent used in the assay. Five levels of liquid, ready-to-use standards, and a BioSystems Gluten Spike Solution are provided with the kit. The spiking solution is intended for the preparation of spiked samples used as recovery controls to verify interference from the matrix and analytical performance in a variety of food and beverage matrixes.

Sample extraction is performed using a proprietary gluten extraction solution specifically formulated by BioSystems. For solid food matrixes, extraction is achieved through a single incubation step lasting 40 minutes at 50 °C. For liquid samples, the extraction process is completed in 10 minutes at room temperature. This simplified extraction protocol ensures effective solubilization of gluten while minimizing processing time and handling complexity. The extracted sample is automatically mixed with the reagent containing the antibody-coated nanoparticles. In the presence of gluten, the interaction between the antigen and antibody induces agglutination of the particles, resulting in the formation of a three-dimensional mesh-like structure that increases the turbidity of the reaction mixture. This increase in turbidity is measured spectrophotometrically at a wavelength of 520 nm. The absorbance is directly proportional to the gluten concentration in the sample and is quantified by interpolation on a calibration curve established using the kit standards. This curve is modeled using a four-parameter logistic (4PL) regression, which enables accurate determination of gluten concentrations across the assay's dynamic range.

As the calibration is based on gliadin concentrations, the analyzer software applies a conversion factor to express the final result as gluten content. Gliadin is generally considered to constitute approximately 50% of the total gluten proteins in wheat. Consequently, the measured gliadin concentration is multiplied by a factor of two to estimate the gluten content.

The BioSystems Y15 analyzer automates all reagent handling steps via a three-axis Cartesian robotic arm. The system processes one test every 60 seconds, delivering an analytical throughput of up to 60 analysis per hour. Results are displayed immediately after analysis, allowing rapid access to gluten quantification data.

CERTIFIED CLAIM STATEMENT: The BioSystems Gluten Immunoturbidimetry method is certified for the quantification of gluten from wheat, barley and rye flours within the scope of Tables 1 and 2.

***Certified method includes**:*

1. BioSystems Y15 automated analyzer

Table 1. Method Performance Claims

				Performance supporting certification[b]			
Matrix	Test Portion	Solvent [a]	Range, mg/kg	Pooled LOD, mg/kg	Pooled LOQ, mg/kg	Recovery, %	RSD_r, %
Corn flour	0.25 g	Aqueous	0-200	1.21	2.40	97.7-226	4.58-28.5
Rice flour	0.25 g	Aqueous	0-200	1.21	2.40	104-160	5.91-10.7
Sausage	0.25 g	Aqueous	0-200	1.21	2.40	100-153	7.82-10.8
Rice cookies	0.25 g	Aqueous	0-200	1.21	2.40	86.7-210	3.70-21.0
Baked cornbread	0.25 g	Aqueous	0-200	1.21	2.40	86.5-111	4.81-10.1
Wine (post-fermentation)	0.25 mL	Aqueous	0-200	1.21	2.40	96.1-103	3.15-4.08

[a] BioSystems Extraction Solution

[b] Performance supporting certification data reported as gluten.

Table 2. Method Selectivity

Commodities	Concentration	No. Commodities Reacting	No. Commodities Interfering
51 Gluten-free products[a]	10 mg/kg	2[b]	0
8 Wheat cultivars[c]	10 mg/kg	8	N/A

[a] Comprising legume products, grains, pseudocereals, tree nut products, animal proteins, fish proteins, fruits, vegetables, non-seed crop materials, binders/emulsifiers/stabilizers, dairy products, beverage product, cocoa product, spice product.

[b] Lupine flour (two sources) and oat flour were positive for gluten.

[c] Including common (114% reactivity), club (48% reactivity), Durum (53% reactivity), einkorn (72% reactivity), emmer (104% reactivity), Khorasan (47% reactivity), spelt (68% reactivity) and triticale (85% reactivity) wheat cultivars.

Table 3. Method History

No.	Date	Summary	Supporting Data
1	July 2025	Original Certification	Certification Report