# INGEZIM GLUTEN. IMMUNOENZYMATIC ASSAY FOR GLUTEN DETECTION USING MONOCLONAL ANTIBODY R5.

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## *1.1. INTRODUCTION*

The Codex Alimentarius Commission is currently discussing setting the maximum level of gluten for “gluten-free foods” at 20 ppm. This pending threshold is expected to be enforced once an official method for gliadin analysis has been established and progress on “toxicity” related to gluten for coeliac patients has been made. Many food companies have already started to include the new threshold in their quality control procedure.
INGEZIM GLUTEN is a quantitative method with a detection limit (1.5 ppm of gliadin) that is well under the forthcoming requirements of the Codex Alimentarius Commission. The R5 antibody used in the detection step is selected for its supreme specificity to the toxic peptide motif. Ready-to-use, European-certified reference standards, recommended by the *Working Group on Prolamin Analysis and Toxicity* (WGPAT)), are included in the kit. In contrast to other similar kits on the market, INGEZIM GLUTEN is also suitable for the quantification of gluten in barley. The performance of INGEZIM GLUTEN in internal validations is presented in this report.

## *1.2. DETECTION METHODS.*

As gluten represents the majority of the cereal proteins and consequently the resulting flours and starches, the first methods described were based on protein measurement using colorimetric methods (Biuret and Lowry methods) and on total nitrogen content (using the Kjedhal method).
Later, more sophisticated methods were developed using high-pressure liquid chromatography (HPLC), sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE), size-exclusion liquid chromatography, capillary electrophoresis and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS). These methods require expensive material and are tedious and cumbersome to use for routine analysis.
Enzyme-linked immunosorbent assays (ELISA) offer good specificity and sensitivity due to the use of antigluten antibodies. This method requires only basic laboratory material and can therefore be used routinely in any laboratory.

## *1.3. REGULATIONS.*

The Codex Alimentarius Commission of the FAO (Food and Agricultural Organization of the United Nations) and the WHO (World Health Organization) has stated that products containing more than 0.3% protein from wheat, rye, barley and oats should be excluded from the coeliac diet. A wheat starch with a 0.3% protein level has an actual gluten content of around 200 ppm (mg/kg) and is declared "gluten-free".
In order to guarantee gluten-free food, the gluten content should be controlled in raw material and finished products. A new maximum level for gluten-free foods has been proposed. In this proposal it is specified that gluten detection in foods and ingredients must be based on an immunological method, offering a detection limit of 10 ppm in products on a dry matter basis. The adaptation of the draft threshold by the Codex Alimentarius Commission will continue once an official method for gliadin analysis has been established and progress on “toxicity” related to gluten for coeliac patients has been made.
The Working Group on Prolamin Analysis and Toxicity (WGPAT) aims to investigate methods and to coordinate research on toxicity of food for individuals with coeliac-sprue disease. Considerable progress has been made in clinical and analytical methods research, including a large collaborative study of the R5 ELISA system for the detection of gliadin, and the development of a certified European reference standard for the quantification of gliadin/gluten in foods.