

## Glutentox® Home

### VALIDATION REPORT

<b>Cat. No.</b>	KIT3010 (5 tests) and KIT3009 (2 tests)
<b>Test name</b>	Glutentox Home
<b>Description</b>	Quick test for the detection of gluten in food, beverages and oral hygiene products.
<b>Sensitivity and Specificity</b>	<p>The limit of detection (LOD) of the assay is 5 ppm of gluten, working at maximum sensitivity (10 drops). The threshold of detection can also be adjusted at 20 ppm (2 drops).</p> <p>This test can specifically detect the presence of the immunotoxic fraction of the prolamins of wheat (gliadin), rye (secalin), barley (hordein) and also of rare varieties of oat (avenin) that can be harmful for celiac patients.</p> <p>However, no positive signal is observed when samples contain vegetal ingredients safe for celiac disease sufferers like rice, corn, soy, buckwheat, sesame, millet, teff, quinoa and amaranth.</p>
<b>Storage</b>	Store at room temperature (15 - 25 °C/ 59 - 77 °F).

#### ASSAY PROCEDURE

- Solid and liquid:

According to manual of use INS3005

#### VALIDATION

##### **Gluten Free food and drinks:**

Tests have been carried out on samples considered gluten-free according to the Codex Alimentarius Commission and the EC Regulation 41/2009 on the composition and labeling of foodstuffs suitable for people intolerant to gluten. Food can be considered as “gluten-free” if its gluten content does not exceed 20 parts per million (ppm\*).

\* Milligrams of gluten per kilo of food (mg/kg).

Food (solid and liquid) Without gluten or labelled gluten-free	Number of spoons	>5ppm	>20ppm
		Threshold	
Cooked ham (gluten-free)	1	negative	negative
Cesar salad sauce (gluten-free)	1	negative	negative
Corn snack (gluten-free)	2	negative	negative
Chocolate milkshake (gluten-free)	1	negative	negative
Yogurt (gluten-free)	1	negative	negative
Fish Surimi (gluten-free)	1	negative	negative
Cheese spread (gluten-free)	1	negative	negative
Soy drink (gluten-free)	1	negative	negative
Semi-skimmed milk	1	negative	negative
Milk with soluble fiber (gluten-free)	1	negative	negative
Rice drink (gluten-free)	1	negative	negative
Corn semolina	2	negative	negative
Corn flour	2	negative	negative
Rice crackers	2	negative	negative
Corn snacks	2	negative	negative
Orange juice	1	negative	negative
Brown rice and corn cracker	2	negative	negative
Canned mashed tomato	1	negative	negative
Muffins (gluten-free)	2	negative	negative
Vegetable cream	1	negative	negative
Soy sauce naturally hydrolyzed	1	negative	negative
Peanut butter	1	negative	negative
Bread/toast (gluten-free)	2	negative	negative
Rice pasta	1	negative	negative
Toothpaste	1	negative	negative
Mouthwash	1	negative	negative
Minced meat	1	negative	negative
Chocolate powder (gluten free)	2	negative	negative
Garlic power	2	negative	negative
Cayenne pepper powder	2	negative	negative
GF Beer (gluten free)	1	negative	negative
Sausage (gluten-free)	1	negative	negative

The food tested above and labelled gluten-free gave negative results at the 5 ppm and 20 ppm thresholds, confirming that they were exempt of gluten and that the test is not giving any false positive in such matrices.

### Products labelled containing gluten:

Tests we performed at the 5 ppm and 20ppm threshold on sample that had ingredients/cereals containing gluten on their label.

Food (solid and liquid) Labelled as containing gluten	Number of spoons	>5ppm	>20ppm
Threshold			
Milk with cereals	1	positive	positive
Spelt drink	1	positive	positive
Spelt crackers	2	positive	positive
Breakfast cereals (with malt extract)	2	positive	positive
Meatballs	1	positive	positive
Meat ravioli	1	positive	positive
Chorizo	1	positive	positive
Chocolate powder (contains wheat flour)	2	positive	positive
Wheat glucose syrup	1	positive	positive

All sample tested gave positive results at both threshold confirming that they had more than 20ppm of gluten. The test has not shown any false negative on common food samples containing gluten.

### Comparative study of gluten content of finished products and ingredients:

Various food samples with different types of matrices were analyzed to quantify or semi-quantify gluten levels. All commercially available food samples were analyzed to determine the correlation between GlutenTox Home, ELISA Sandwich G12 and ELISA Sandwich R5 (REF 1). The following data were obtained:

	Sample	Glutentox Home (2 drops)	ELISA Sandwich G12	ELISA Sandwich R5
1	Corn starch	<20ppm	16.3 ppm	21.5 ppm
2	Cream	< 20 ppm	<3 ppm	<3 ppm
3	BBQ spices	>20 ppm	23.3 ppm	19.2 ppm
4	Paprika	<20 ppm	<3 ppm	<3 ppm
5	Wheat Starch	>20 ppm	166 ppm	203 ppm
6	Strawberry flavor	<20 ppm	12 ppm	14.6 ppm
7	Pudding	>20 ppm	36 ppm	28.6 ppm
8	Ham flavor	< 20 ppm	<3 ppm	<3 ppm
9	Glucose syrup	>20 ppm	256 ppm	244 ppm

10	Rice milk	>20 ppm	80 ppm	68.2 ppm
11	Sausage 1	>20 ppm	113 ppm	98.2 ppm
12	Sausage 2	>20 ppm	100 ppm	155 ppm
13	Cured pork loin	< 20 ppm	<3 ppm	<3 ppm
14	Hamburger meat	>20 ppm	96 ppm	89 ppm
15	Cake (gluten-free)	< 20 ppm	<3 ppm	<3 ppm
16	Aperitive snacks	< 20 ppm	<3 ppm	<3 ppm
17	Baby food	>20 ppm	96 ppm	105 ppm
18	Biscuit (gluten-free)	< 20 ppm	<3 ppm	<3 ppm
19	Bread (gluten-free)	< 20 ppm	<3 ppm	<3 ppm
20	Chocolate	< 20 ppm	<3 ppm	3.2 ppm
22	Ice cream	< 20 ppm	<3 ppm	<3 ppm

GlutenTox Home can accurately discriminate sample that are considered gluten-free (20 ppm) or not (>20 ppm). The test showed 100% correlation with the food sample tested with the above 3 methods. No false positive or false negative were observed.

#### Gluten-free samples spiked (artificially contaminated) with known levels of gluten:

Food matrices considered gluten-free were artificially contaminated with a gluten to obtain concentrations equivalent to 20ppm and 10 ppm. All tests have been carried out by preparing the samples and analyzing them (n = x) according to the kit insert.

Food (solid and liquid) gluten-free *contaminated with gluten	Number of spoons	Spiked/contaminated with	>5ppm (10 drops)	>20ppm (2 drops)
			Threshold	
Cooked ham (gluten-free)*	1	20ppm	positive	positive
Caesar salad sauce (gluten-free)*	1	20ppm	positive	positive
Corn cracker (gluten-free)*	2	20ppm	positive	positive
Corn cracker (gluten-free)*	2	10ppm	positive	negative
Chocolate milkshake (gluten-free)*	1	10ppm	positive	negative
Yogurt (gluten-free)*	1	10ppm	positive	negative
Fish Surimi (gluten-free)*	1	10ppm	positive	negative
Cheese spread (gluten-free)*	1	10ppm	Faint positive	negative
Soy drink (gluten-free)*	1	10ppm	positive	negative
Semi-skimmed milk	1	5ppm	Faint positive	negative

**Conclusion:**

The results of this validation study show that despite the simplicity of the method (short and user-friendly), the results are consistent, with no discrepancies and good correlation within a large variety of food matrices. No false positive or false negative have been observed with this method. It is also a good tool to discriminate between samples that have gluten below 5 ppm for celiac patients that are very sensitive to gluten.

Note 1: Different studies demonstrate that foods containing low amounts of polyphenols or tannins, (e.g. samples with less than 70% of chocolate, black tea, coffee, wine, berries, etc.), samples with antioxidants, such as vitamins A, E and C and food heat-treated with temperatures above 180 °C (356 °F) should be tested under conditions of maximum sensitivity, 5ppm (10 drops) (see INS3005).

Note 2: GlutenTox Home is not recommended for food samples with a high content of polyphenols and tannins, i.e. foods in which the major component (> 70%) is chocolate, black tea, coffee, wine, berries, etc.; in these cases, the extraction process can be insufficient and therefore underestimate the amount of gluten in the sample.

**References:**

C.Torgler, Analytical tools to detect gluten immunotoxic fractions in food based on monoclonal antibodies raised against the 33-mer peptide. Proceedings of the 24<sup>th</sup> Meeting Working Group on Prolamin Analysis and Toxicity (PWG).