GlutenTox® Sticks Plus

Quick test for the determination of gluten content

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Quick test for the determination of gluten content

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1. Purpose

GlutenTox® Sticks Plus is an immunochromatographic test for the determination of immunotoxic gluten in food, beverages, other consumer products and on working surfaces, which are harmful for celiac patients.

2. Introduction

Celiac disease affects the small gut and provokes atrophy of intestinal villi that interferes with the absorption of nutrients such as proteins, fat, carbohydrates, mineral salts, and vitamins. Celiac disease is due to an inappropriate immune system response to gluten (a mix of proteins present in some grains) from wheat, rye, barley and, to a lesser extent, oat [ref. 1 and 2]), leading to diarrhea, vitamin and mineral deficiencies, anemia and thin bones (osteoporosis). Celiac disease affects people of all ages.

Currently, the only treatment for celiac disease sufferers is a strict gluten-free diet during their entire lifetime, which presents great difficulties because gluten, in addition to being present in many foods, may also be found in food additives and preservatives.

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.

3. Test basis

GlutenTox® Sticks Plus is an immunochromatographic test for the determination of gluten in all kind of foodstuffs, from raw materials to processed food and/or heat-processed food, beverages and also other consumer products.

GlutenTox® Sticks Plus can also be used for surface detection to ensure the surfaces are suited to produce gluten-free products. This rapid test is useful in routine monitoring of gluten to ensure that products comply with a program of Hazard Analysis and Critical Control Points (HACCP), and to ensure proper labeling. It also allows quick decisions and corrective actions in case there is any risk of contamination along the production chain.

In all methods used for gluten analysis in a given sample, the gluten first has to be extracted from the sample’s matrix. Extraction is one of the most critical points of the testing process. The extraction solution provided in this kit, Universal Gluten Extraction Solution (UGES), is suited for all types of food thanks to the combination of denaturing agents, reducing agents and solubilizers.

For the analysis of food containing polyphenols, tannins such as chocolate, tea, coffee, wine, purple corn and corn fiber, soy, berries, etc., it is necessary to add a special additive in the extraction process. This additive acts as a chelating agent of proteins, preventing the interference of the above mentioned compounds in the extraction process (see Figure 1). The same applies in the case of cosmetic products with antioxidant compounds such as vitamins A, C and E, carotenes, carotenoids, etc.
After the extraction, during the detection step, the sample reacts with the colored conjugates anti-gliadin antibody [ref. 3], previously placed in the stick. This complex spreads by capillarity through the sticks and reacts with the anti-gliadin antibody, also previously immobilized on the strip. If the result is positive, a RED line appears in the result zone of the stick. The absence of the RED line indicates a negative result. Whether or not gluten is present, the mix of the conjugate moves through the stick up to the control region where antibodies have been immobilized and if the test was properly performed a BLUE line (control line) will appear. The appearance of this BLUE line is used: 1) to verify that the volume of sample that was added was sufficient, 2) that the flow has been appropriate, and 3) as an internal control of the reagents. If the BLUE line does not appear, the test should be considered invalid.

**Figure 1. Scheme of use for GlutenTox® Sticks Plus**

This technique allows two options for the reading of the results: a visual reading and a digital reading with the GlutenTox® Reader scanner (see Annex). The visual reading allows semi-quantitative results; for quantitative results with high sensitivity, GlutenTox® Reader is required. GlutenTox® Reader combines a highly sensitive optical detector, an integrated electronic system and an effective data processing system. It is CE labeled and produced according to ISO 9001 and ISO 13485.
4. Storage conditions and stability
To obtain optimal test performance, GlutenTox® Sticks Plus must be stored in its original packaging, between 2 and 30°C and used before expiration date printed on the label.

Warning: The tube with the sticks should not be opened until the time of use. Once the seal is broken, keep the tube with the sticks at room temperature. To avoid water condensation, do not refrigerate the tube after opening. Never freeze.

5. Precautions
- Only for testing food, beverages, other consumer products and surfaces.
- Do not ingest any solutions (liquids) and/or additive of the kit.
- Do not use after expiration date.
- The use of non-powdered disposable gloves is recommended.
- Only touch sticks with gloves or washed hands and do not touch the lower absorbent part.
- A negative result does not exclude that the food contains gluten, since it can be distributed unevenly or be below the detection limit.

6. Supplied materials
- GlutenTox® Sticks (25 sticks).
- Universal Gluten Extraction Solution (UGES) (250 mL).
- Dilution Solution (30 mL).
- Microtiter plate strips (4 strips x 8 wells).
- Positive Control (chickpea flour contaminated with gluten, 10 g).
- Negative Control (corn flour, 10 g).
- Instructions for use.

7. Necessary materials not supplied
- Analytical scale (accurate to 0.1 g).
- Closing test tubes (>10 mL).
- Test vials.
- Pipettes and tips.
- Vortex agitator (optional).
- Wheel agitator.
- Thermostatic bath (not necessary for non-heat-processed samples with simple matrix composition).
- Centrifuge (optional).
- Disposable gloves.
- Distilled water.
- Watch/chronometer.

For **food containing polyphenols or tannins, and cosmetics containing antioxidants**, the following additional materials, available in the catalog of Biomedal*, (KT-5320) are required:

- Special polyphenol additive (25 g).
- Positive Control containing polyphenols (cocoa powder with gluten, 10 g).
- Negative Control containing polyphenols (gluten-free cocoa powder, 10 g).

  ! **NOTE:** Foods rich in polyphenols or tannins are: chocolate, tea, coffee, wine, purple corn and corn fiber, soy, berries, etc.

  ! **NOTE:** The most common antioxidants in cosmetic products are vitamins A, C and E, carotenes, carotenoids, etc.

* For more information contact your supplier.
8. Sample preparation (food, beverages and other consumer products)

8.1. Solid samples

1. Homogenize, mill and/or triturate the sample.

2. Weigh 1 g of sample in a test tube.

   ! NOTE: If the sample, solid or liquid, contains polyphenols, tannins (e.g. chocolate) or antioxidants, weigh and add 1.0 g of special polyphenol additive to the sample tube.

3. Add 10 mL of Universal Gluten Extraction Solution (UGES). Close the tube and mix to homogenize (for example, using a vortex agitator). For provided positive and negative controls, perform the same procedure.

4. Depending on the complexity of the sample matrix and whether the food sample has been processed by heat or not, follow one of the 3 options below (see Figure 2):

   a) Non-heat processed samples with simple matrix composition

       Incubate the sample at room temperature for 40 minutes with a wheel agitator.

   b) Heat-processed samples and/or with complex matrix composition

       Incubate the sample 50ºC in a waterbath for 40 minutes, shaking the tube periodically by tipping it over or using a vortex agitator.

   c) Samples containing polyphenols, tannins or antioxidants

       Incubate the sample 50ºC in a waterbath for 40 minutes, shaking the tube periodically by tipping it over or using a vortex agitator.

       ! NOTE: If the type of sample is difficult to determine, we recommend following Option b (Heat-processed sample and/or with complex matrix composition) to facilitate the extraction.

5. Clarify the sample to eliminate solids by decanting or centrifuging. Solid parts can alter the results.

6. Transfer the clarified mixture to a clean tube.

   ! NOTE: Once extracted, the samples must be analyzed as quickly as possible.
8.2. Liquid samples

**NOTE:** Liquid samples with polyphenols, tannins or antioxidants have to be analyzed according to the point 8.1 solid samples.

Liquid samples such as milk, juices, soft drinks, organic drinks (soy, rice, oat, spelt drinks), beers and broths do not require intensive extraction, manually shaking of 1 or 2 minutes is sufficient, and does not require the centrifugation or decantation step.

1. Add 1 mL of sample in a test tube.
2. Add 9 mL of Universal Gluten Extraction Solution (UGES). Close the tube and mix to homogenize (for example, using a vortex agitator).
3. Shake the sample for 1-2 minutes manually or using a vortex.

9. Test implementation for extracted samples

1. Bring the extracted samples, controls, the Dilution Solution, and the tube with the GlutenTox® Sticks to room temperature (20-26°C degrees).

2. Dilute the sample with Dilution Solution in test tubes or vials. A maximum volume of 900-1000 µL is sufficient to perform the test.
7

**Table 1. Dilutions table**

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Gluten Detection Limit (ppm)</th>
<th>Volumen of extracted sample (µL)</th>
<th>Volumen of dilution (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10</td>
<td>&gt; 1</td>
<td>100</td>
<td>900</td>
</tr>
<tr>
<td>1:40</td>
<td>&gt; 3</td>
<td>25</td>
<td>975</td>
</tr>
<tr>
<td>1:100</td>
<td>&gt; 10</td>
<td>10</td>
<td>990</td>
</tr>
<tr>
<td>1:250</td>
<td>&gt; 20</td>
<td>4</td>
<td>996</td>
</tr>
<tr>
<td>1:500</td>
<td>&gt; 40</td>
<td>2</td>
<td>998</td>
</tr>
<tr>
<td>1:1000*</td>
<td>&gt; 100</td>
<td>1</td>
<td>999</td>
</tr>
</tbody>
</table>

*Serial dilutions can be done for higher accuracy: 5 µL of sample in 495 µL of dilution solution (dilution 1:100) and then, 10 µL of this dilution in 990 µL of dilution solution (dilution 1:10). Finally, a 1:1000 dilution is obtained.

If the expected amount of gluten in the sample is unknown, we recommend testing with the lowest dilution (i.e., dilution 1:10) allowing maximum sensitivity. In case of a positive result (appearance of RED line), the test can be repeated with a greater dilution for a semi-quantitative estimation of the gluten concentration in the sample (see section 13 "Analytical Features").

**Note:** In samples with polyphenols, tannins or antioxidants, in which the special polyphenol additive is added in the extraction step, the minimum dilution admitted is 1:40. Never perform at lower dilutions (i.e., dilution 1:10), since the presence of special polyphenol additive affects the proper performance of the stick if the dilution is less than 1:40.

**Note:** In sample with high levels of fat, avoid taking the upper layer that contains the fat.

**Note:** The diluted samples must be analyzed as quickly as possible and the remaining material should be discarded.

3. Place 100 µL of the diluted sample in a well of the microtiter strip supplied in the kit.

4. Open the tube with GlutenTox® Sticks, take out the number of sticks necessary and close the tube immediately.

5. Introduce the stick vertically in the well with the diluted sample.

6. Wait **10 minutes** and read the result on the stick.

**Note:** When planning to use GlutenTox® Reader, a dilution 1:10 of the sample should be performed. Wait at least 30 minutes (and not more than 2 hours) before introducing the stick to the Reader.
10. Surface Analysis

1. Bring the Dilution Solution and the tube with the GlutenTox® Sticks to room temperature (20-26°C degrees).

2. Rub the cotton wool side of the stick against five areas of 1 cm² or along a line of 5 cm (see Figure 4).

3. Place 100 µL of the Dilution Solution in a well of the microtiter strip supplied in the kit.

4. After the stick has been rubbed on the surface to be analyzed, introduce it vertically into the well.

5. Wait 4 minutes and read the result on the stick.

6. Wait 10 minutes and read the result on the stick.

Figure 4. Procedure for surface analysis
11. Interpretation of results

NEGATIVE: A single BLUE line (control line) appears in the central part of the stick (control zone).

POSITIVE: In addition to the control line (BLUE), a RED line (result line) appears in the result zone.

**NOTE:** The intensity of the red line in the results zone will vary depending on the gluten concentration present in the sample.

INVALID: The control line (BLUE) does not appear, whether or not the result line appears (RED). The most common causes for the appearance of an invalid result are: an insufficient quantity of sample, following an incorrect procedure, or deterioration of the reagents. In the case of invalid results, it is necessary to revise the procedure and repeat the experiment with a new test. If the problem persist, please contact the supplier.

12. Quality control

Internal procedural quality control is included in the test. The blue line that appears in the control zone is the internal control of the process, checking that the sample volume is sufficient and that the procedure that has been followed is adequate. To make sure the kit is being used properly, use the Positive and Negative Control included in the kit. Use this control tubes following the instructions from the point 8.1.2. The results that you must obtain are: a positive result with the Positive Control and a negative result with the Negative Control.

13. Analytical features

Different assays have been carried out to characterize the main analytical parameters of the test: sensitivity and specificity.

**Sensitivity**

The detection limit of GlutenTox® Sticks Plus is 15 ng/mL of gliadin. This value was obtained using different solutions of known concentrations of gliadin in the dilution solution.

Food, beverages and other consumer products

As regards ppm of gluten, the detection limit of the test will depend on the dilution made with the sample once extracted.

The following table outlines the dilution to be carried out according to the level of gluten to detect.

<table>
<thead>
<tr>
<th>Result of the test</th>
<th>1:10</th>
<th>1:40</th>
<th>1:100</th>
<th>1:250</th>
<th>1:500</th>
<th>1:1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>&gt; 1 ppm</td>
<td>&gt; 3 ppm</td>
<td>&gt; 10 ppm</td>
<td>&gt; 20 ppm</td>
<td>&gt;40 ppm</td>
<td>&gt;100 ppm</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt; 1 ppm</td>
<td>&lt; 3 ppm</td>
<td>&lt; 10 ppm</td>
<td>&lt; 20 ppm</td>
<td>&lt; 40 ppm</td>
<td>&lt; 100 ppm</td>
</tr>
</tbody>
</table>

Table 2. Detection limit
Surface analysis
The result obtained with the test indicates the presence or absence of gluten on the analyzed surface; it cannot be extrapolated into any value of gluten in ppm.

If by analyzing five areas of 1 cm$^2$ or a line of 5 cm a minimum of 40 ng/cm$^2$ is detected, it can be “estimated” that for an analyzed working surface of 1000 cm$^2$ (40 cm x 25 cm), working with a food mass of 1 Kg, the final product will have less than 0.04 ppm (0.04 mg gluten/Kg of food). This quantity is about 500 times less than the quantity recommended by the European norms established at 20 ppm (20 mg gluten/Kg of food).

This means that the method has a large safety margin and that its use provides a guarantee to clients, celiac associations and food safety inspectors [ref. 4].

Specificity
This test can specifically detect the presence of the toxic fraction of the prolamins of wheat (gliadin), rye (secalin), barley (hordein) and as well varieties of oat (avenin) that can be toxic and that can therefore be harmful for celiac patients. However, when the samples contain rice, corn, soy, buckwheat, sesame, millet, teff, quinoa and amaranth, no positive signal is observed, as these vegetal ingredients are safe for celiacs.

Internal Validation
To ensure the test’s ability to analyze all types of food (of diverse nature) and other samples such as cosmetics and personal care products, different commercial samples have been tested. After analyzing the samples with GlutenTox® Sticks in all types of matrices tested (see Table 3 and 4) the results were satisfactory and consistent with the gluten found with the approved method of Codex Alimentarius, which demonstrates the applicability of the test on a broad range of samples.
<table>
<thead>
<tr>
<th>Group</th>
<th>Tested samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour and semolina</td>
<td>Corn flour*, precooked corn flour, corn semolina, rice flour, wheat flour, buckwheat flour</td>
</tr>
<tr>
<td>Milk products</td>
<td>Cow milk, milk with soluble fiber, milk with cereals, flavoured or natural yogurt*, cheese spread, shredded cheese blend</td>
</tr>
<tr>
<td>Baked and cereal products</td>
<td>Toast, bread stick*, biscuits (Rich tea), chocolate cookies, Madeleine, cake, cornflakes, pastas, corn pancakes, rice cakes, spelt cake, snacks</td>
</tr>
<tr>
<td>Meat products</td>
<td>Minced turkey, minced chicken, turkey sausage, chicken nuggets, pork sausages, chorizo, pork liver pâté*</td>
</tr>
<tr>
<td>Fishery products</td>
<td>Cod and Hake</td>
</tr>
<tr>
<td>Vegetables</td>
<td>Lettuce mix, fried vegetables</td>
</tr>
<tr>
<td>Broth, soups, creams and dry mixes</td>
<td>Vegetable broth, chicken rice soup, dehydrated vegetable soup, stock cubes, vegetable soup, peanut butter</td>
</tr>
<tr>
<td>Sauces, dressing, spices and condiments</td>
<td>Yogurt salad dressing, ketchup, soy sauce, salad dressing, garlic powder, paprika powder, cooking cream</td>
</tr>
<tr>
<td>Sugars</td>
<td>Glucose syrup, powdered sugar</td>
</tr>
<tr>
<td>Prepared meals and dishes</td>
<td>Meatballs in sauce with peas, Meat Ravioli in Egg Dough, bean stew</td>
</tr>
<tr>
<td>Fatty foods</td>
<td>Olive oil, sunflower oil, butter, margarine, cream</td>
</tr>
<tr>
<td>Acidic foods</td>
<td>Tomate sauce, wine vinegar, apple cider vinegar, lemon juice</td>
</tr>
<tr>
<td>Beverages</td>
<td>Water, milk, fruit juices, beer, soy drinks, rice drinks, oat drinks, soft drinks</td>
</tr>
<tr>
<td>Oral hygiene products</td>
<td>Toothpaste, mouthwash</td>
</tr>
</tbody>
</table>

*also validated at 1 ppm
Table 4. Other samples tested for validation of GlutenTox® Sticks Plus

<table>
<thead>
<tr>
<th>Group</th>
<th>Tested samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal care products</td>
<td>Bath gel, shampoo, deodorant, toothpaste, mouthwash</td>
</tr>
<tr>
<td>Cosmetics</td>
<td>Creams (face, body and hands), cleanser, lip balm</td>
</tr>
<tr>
<td>Others</td>
<td>Pet food (dry food, wet food), cleaning products, drugs (tablets, capsules and syrups)</td>
</tr>
</tbody>
</table>

14. References


3. Moron B., et al.; "Sensitive detection of cereal fractions that are toxic to celiac disease patients by using monoclonal antibodies to a main immunogenic wheat peptide", 2008; 87:405-414.

The Reader is designed for the exclusive gluten determination with GlutenTox® Sticks Plus.

Follow the instructions for the sample preparation and analysis as explained in the points 8 and 9 of this manual. Introduce the data sample into the Reader once the incubation step is done, by inserting the stick in the stick holder and then into the Reader. The reading of the stick will provide quantitative result of the gluten content of the sample. A graphic scheme of this procedure is given below.

1) Introduction of the stick into the stick holder and in the Reader.

2) When using the Reader in stand-alone mode enter the identification data of the sample in the device and press "measure" to start the reading.

3) After the scanning of the stick, the results will be displayed on the screen.

4) When using the GlutenTox® Reader while plugged into a computer, use the provided software to identify the sample and start the reading.

! **NOTE:** More details about the use of the Reader is provided in the Manual of use of GlutenTox® Reader.
For more information, please, visit our website or contact us:

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