GlutenTox® SticksPlus for Cube

GlutenTox® Sticks Plus for Cube

Quick test for the quantification of gluten content in food and beverages with the Hygiena® Cube.

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1. Intended Purpose

GlutenTox® Sticks Plus for Cube is a rapid immunochromatographic test for the quantification of gluten, which is harmful to celiac patients, in food, beverages and other consumer products.

2. Introduction

Celiac disease is a disorder that damages the small intestine causing the atrophy of the intestinal villi, which interferes with the absorption of nutrients such as proteins, lipids, carbohydrates, mineral salts and vitamins. This disease is caused by an inappropriate response of the immune system to gluten (a mix of proteins found in cereals) from wheat, barley, rye, and to a lesser extent, from 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, lifelong gluten-free diet that 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 “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 for Cube is an immunochromatographic (lateral flow) test for the quantification of gluten in foods with different compositions and levels of processing, from raw materials to heat-processed food, beverages and other consumer products. It is based on the anti-gliadin G12 antibody, which specifically recognizes the 33-mer peptide, the most immunogenic fraction of gluten [ref. 3]. This rapid test is useful in routine monitoring of gluten presence to guarantee 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 must 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, including tannins, such as chocolate, tea, coffee, wine, purple corn and corn fiber, soy, berries, legumes like chickpeas and lentils, etc., it is necessary to use a special additive (not included in this kit, see section 5) that prevents 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 gluten present in the sample reacts first with the anti-gliadin G12 antibody [ref. 3] conjugated to red-colored particles, previously placed on the stick. The resulting complexes spread by capillarity through the stick and react with a second anti-gliadin antibody, also previously immobilized on the stick. If the result is positive, a RED line appears in the Test Zone (T) of the cassette. The absence of the RED line indicates a negative result. Whether or not gluten is present, the sample moves through the stick up to the Control Zone (C) where, if the test was properly performed, a BLUE line will appear, due to the accumulation of blue-colored particles also included in the stick.

The presence of this BLUE line indicates that: 1) the sample volume was enough, 2) the sample flow was appropriate, and 3) the conjugate particles included in the test were properly released. If the BLUE line does not appear, the test should be considered invalid.

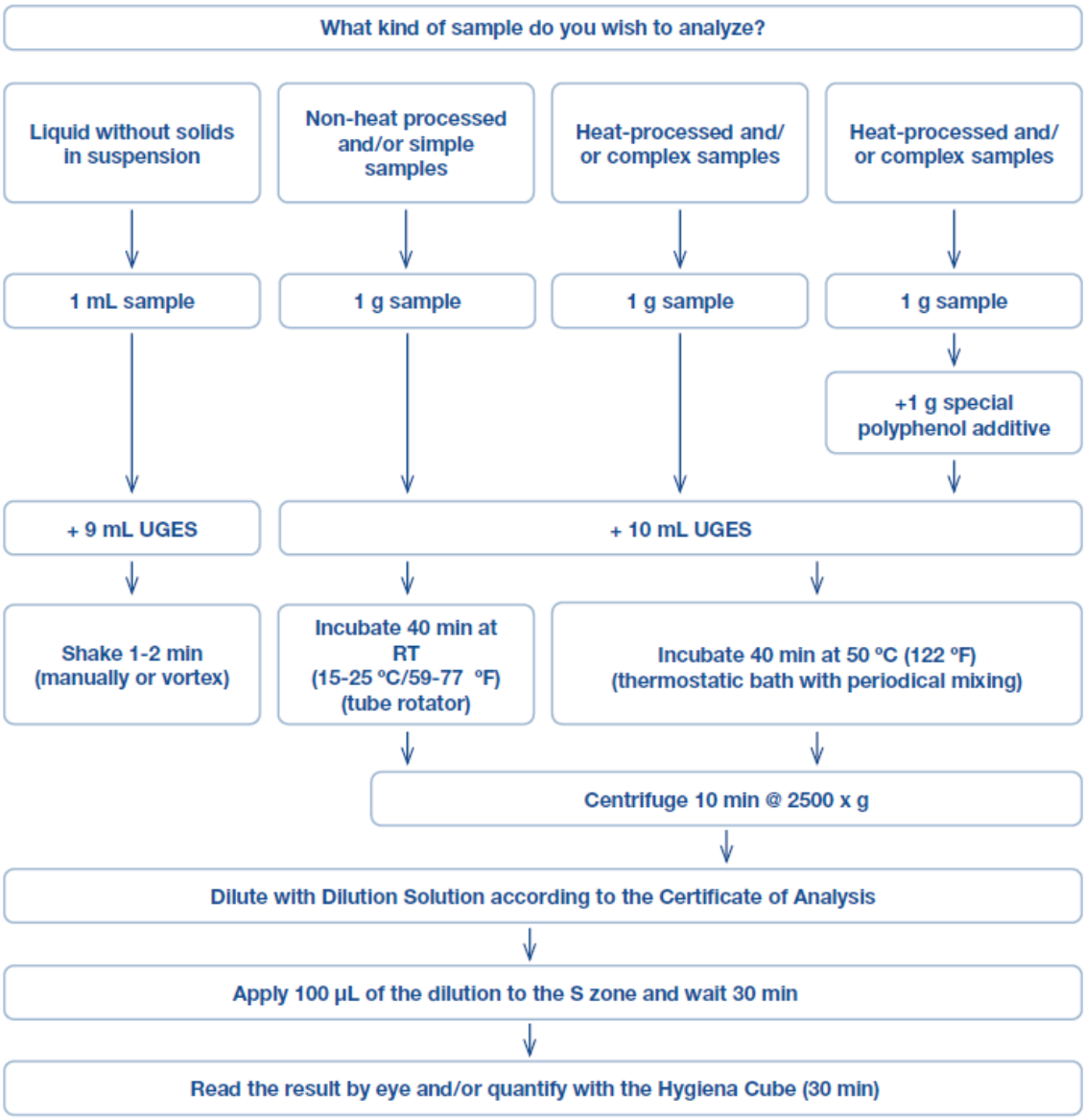

Figure 1. Scheme of use for GlutenTox Sticks Plus for Cube

This test allows two options for the reading of the results: visual or/and quantitative evaluation with the Hygiena Cube. The Hygiena Cube is CE labeled and produced according to ISO 9001 and ISO 13485.

4. Supplied materials

- GlutenTox cassettes (12 cassettes)
- Universal Gluten Extraction Solution (UGES) (125 mL)
- Dilution Solution (15 mL)
- RFID Card to use with Hygiena Cube
- Instructions for use
- Download instructions from the website

5. Necessary materials not supplied

- Analytical scale (accurate to 0.1 g)
- Thermostatic bath (not necessary for non-heat-processed samples with simple matrix composition)
- Capped centrifuge test tubes (>10 mL)
- Test vials (1.5 – 2 mL)
- Centrifuge (optional)
- Pipettes and disposable tips
- Disposable gloves
- Vortex mixer (optional)
- Watch/chronometer
- Tube rotator
- Polyphenol Pack (KIT3008)*
  Pack (Kit) contains:
  1. Special polyphenol additive (25 g)
  2. Positive Control with polyphenols (cocoa powder + gluten, 10 g)
  3. Negative Control with polyphenols (gluten-free cocoa powder, 10 g)

*Required for foods rich in polyphenols or tannins such as chocolate, coffee, tea, wine, purple corn and corn fiber, soy, berries, legumes (chickpeas, lentils), etc.
*Required for antioxidant-containing cosmetics, i.e., those with Vitamin A, C and E, carotenes, carotenoids, etc.

6. Storage conditions and stability

For optimal test performance, GlutenTox Sticks Plus for Cube must be stored in its original packaging, between 15 - 25 °C (59 - 77 °F) and used before the expiration date printed on the label.

Note: The envelopes with the cassettes should not be opened until the time of use. Never freeze.

7. Precautions

- Only for testing food, beverages and other consumer products.
- Do not ingest the kit solutions (liquids) or any kit components
- Do not use after the expiration date.
- The use of non-powdered disposable gloves is recommended.
- It is recommended to analyze samples in duplicate.
- Manipulate the cassettes with gloves or washed hands and do not touch the Sample Zone (S) within the cassette to avoid accidental contamination of the test with gluten traces.
- If a sample is heterogeneous (e.g., a salad), make sure to take a representative part of each ingredient and mix them to make a homogenous sample. If the gluten in the sample is unevenly distributed and you do not do this, a false negative could be obtained.

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 and add it to a test tube.

   a. If the sample, solid or liquid, contains polyphenols, tannins (e.g., chocolate) or antioxidants, weigh and add 1 g of special polyphenol additive to the sample tube and mix it vigorously to achieve complete homogenization of the mixture.

3. Add 10 mL of Universal Gluten Extraction Solution (UGES). Close the tube and mix to homogenize (for example, using a vortex mixer).

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 2 options below (see Figure 2):

   Option A: Non-heat processed samples with simple matrix composition:
   Incubate the sample at room temperature (15 - 25 °C / 59 - 77 °F) for 40 minutes with a tube rotator.

   Option B: Heat-processed samples, difficult-to-determine samples or those with complex matrix compositions:
   Incubate the sample at 50 °C (122 °F) in a water bath for 40 minutes, shaking the tube periodically by tipping it over or using a vortex mixer.

5. Allow separation of solids by settling or centrifugation (10 min at 2,500 x g). Solid parts can alter the results.

6. Transfer the clarified supernatant to a clean tube. Once extracted, the sample must be analyzed as quickly as possible.

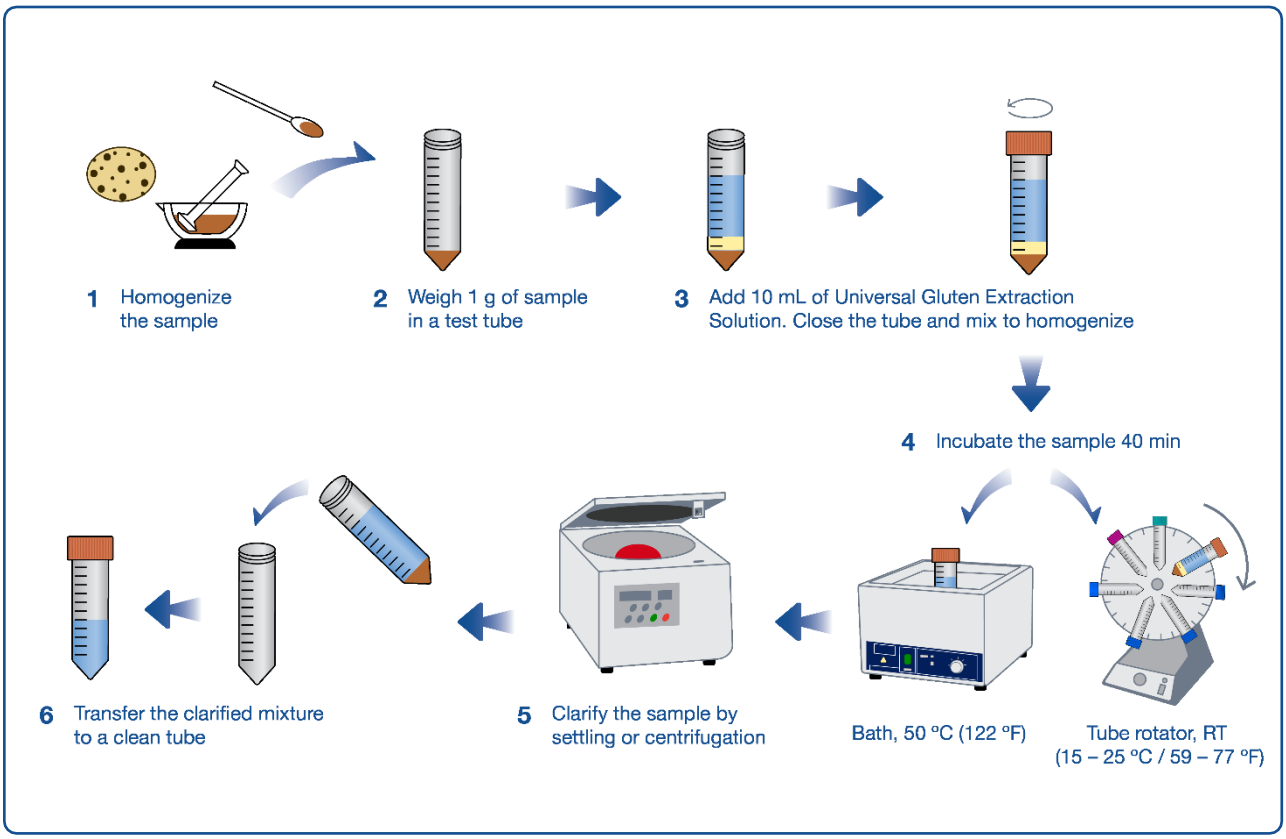

Figure 2. Scheme of the extraction procedure of the solid samples

8.2 Liquid samples

Liquid samples such as milk, juices, soft drinks, organic drinks (soy, rice, oat, spelt drinks) and broths do not require intensive extraction. For this reason, manually shaking for 1 or 2 minutes is sufficient and the extracts do not require a centrifugation or settling step.

1. Add 1 mL of sample to a test tube.
    a. If the sample contains polyphenols, tannins (e.g., chocolate) or antioxidants, weigh and add 1 g of special polyphenol additive to the sample tube and mix it vigorously to achieve complete homogenization of the mixture.
2. Add 9 mL of Universal Gluten Extraction Solution (UGES) and close the tube cap tightly.
3. Shake the sample for 1-2 minutes, manually or using a vortex mixer.

9. Test implementation for extracted samples

1. Bring the extracted samples, controls, Dilution Solution and cassettes to room temperature (15 - 25 °C / 59 - 77 °F).
2. Dilute the extracted samples with Dilution Solution in test tubes or vials.
   Note: if samples contain high levels of fat, avoid taking the upper layer containing the fat.
   The appropriate dilution corresponding to the batch of GlutenTox cassettes **can be found in the Certificate of Analysis included in each kit**. A final volume of 900 - 1000 µL is sufficient to perform the test.

3. Open the envelope of the cassette. If you wish to perform positive and negative controls, you will need one cassette for each control. Add 100 µL of the diluted sample into the Sample Zone (S) of the cassette.

4. Wait 30 minutes and read the result on the cassette (see section 11).

5. To quantify the gluten in the sample, wait 30 minutes and analyze the cassette with the Hygiena Cube (do not wait more or less than 30 minutes, read at 30 minutes only).

6. It is recommended to analyze the samples in duplicate to obtain conclusive results. Diluted samples must be analyzed as quickly as possible and the remaining material should be discarded.

   Do not analyze the cassette with the Hygiena Cube if:

   - Points appear in the Test Zone (T) of the cassette (in the central area or at one of the ends) instead of a homogeneous line
   - The test and control lines appear displaced from the Test (T) and Control (C) Zones of the cassette, respectively.

10. Use of the Hygiena Cube

The cube is designed for the quantification of gluten content with GlutenTox Sticks Plus cassettes. The quantification is from 3 ppm to 40 ppm gluten. Note: Before using, please carefully read the Hygiena Cube instructions and the Hygiena Cube DataReader Software manual.

To quantify your sample, follow these steps:

a) Using the Hygiena Cube in stand-alone mode.

1. Turn on the device by pressing the button briefly (< 1 sec). The display shows "ON".
2. 30 minutes after adding the sample to the cassette, place the test into the adapter. Be sure that the test is correctly placed in the adapter.
3. Place the Hygiena Cube on top of the adapter and test. Ensure that the Hygiena Cube is positioned correctly.
4. Press the button briefly. The display will show "RFID TEST". Put the lot specific RFID card onto the top of the device.
5. The display shows "TEST". Press the button briefly. The display shows "RUN".
6. The result is displayed on the screen.
7. Press the button briefly to return to step 1.

You <u>cannot</u> use Timer Measurement mode.

b) Using the Hygiena Cube in remote measurement mode.

1. Turn on the device by pressing the button briefly (< 1 sec). The display shows "ON".
2. Connect it to the PC via the provided USB cable.
3. Open the Hygiena Cube DataReader Software. Check that the Hygiena Cube is successfully connected.
4. 30 minutes after adding the sample to the cassette, place the test into the adapter. Be sure that the test is correctly placed in the adapter.
5. Place the Hygiena Cube on top the adapter and test. Be sure that the Hygiena Cube is correctly placed.
6. Click on the “Start Measurement” button.
7. Different windows will appear depending on the Setup configuration selected previously.
8. Put the lot-specific RFID card on the top of the device. Press "OK".
9. The measurement will start immediately.
10. The results will be added to the list and the 2D volume diagram will be presented.

You cannot use Timer Measurement mode.

IMPORTANT NOTE!
When the gluten content in the sample is less than 3 ppm (or when the sample is gluten-free) the result on the Hygiena Cube will appear as <3 ppm and <LOQ (Limit of Quantification); however, if the sample has traces of gluten (<3 ppm), these could be visualized as a faint red line in the Test Zone (T) of the cassette.

11. Visual interpretation of results

For quantification with the Hygiena Cube, see section 10. For visual interpretation, the results can be:

NEGATIVE: If, after 30 minutes, a single BLUE line (control line) appears in the Control Zone (C) of the cassette. The sample contains less than 3 ppm of gluten.

POSITIVE: If after 30 minutes, in addition to the control line (BLUE), a RED line (results line) also appears in the Test Zone (T) (this red line may appear before 30 minutes). The sample (*) could contain more than 3 ppm of gluten and be quantifiable with the Hygiena Cube (Note: if the cassettes are invalid or not suitable for quantification, do not analyze them).

(*) It may happen that the RED line in the Test Zone (T) is faint and the result in the Hygiena Cube is <LOQ. This means that the sample contains less than 3 ppm of gluten; it contains traces of gluten that cannot be quantified with the Hygiena Cube.

INVALID AND/OR NOT SUITABLE FOR QUANTIFICATION:

When the control line (BLUE) does not appear, regardless of whether the result line (RED) appears or not.

If points appear in the Test Zone (T) of the cassette (in the central zone or at one of the ends) instead of a homogeneous line, and/or if the test and control lines appear displaced from the Test (T) and Control (C) Zones of the cassette, respectively.

The most common causes for which an invalid and/or not suitable for quantification result may appear are an insufficient volume (<100 µL) of sample in Zone S, an error during the procedure, or a deterioration of the reagents or a cassette defect. If this occurs, the procedure should be revised and the test repeated with a new test cassette. If the problem persists, you should contact your provider.

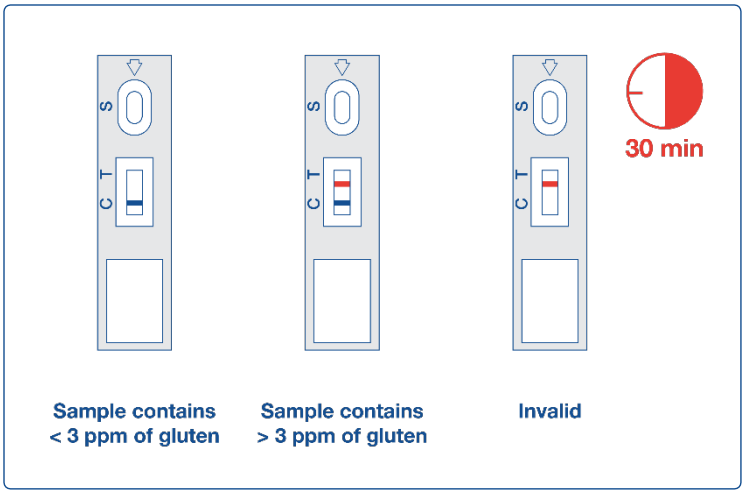

Figure 3. Visual interpretation of results

12. Quality control

Internal procedural quality control is included in the test. The blue line in the Control Zone (C) is a built-in feature that indicates both a sufficient volume and a correct flow of the sample, with proper release of the conjugate particles. In addition, Positive and Negative Controls can be used, according to the instructions beginning in Section 8.1.2, to confirm a correct test performance; these control materials must provide clear positive and negative results, respectively, if the test procedure was conducted properly.

13. Analytical features

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

Sensitivity

The quantification limit of GlutenTox Sticks Plus for the Cube is 3 ppm gluten. This value was obtained by analyzing contaminated samples with wheat flour of known concentration.

Note: To precisely quantify gluten in a sample, please use GlutenTox ELISA Rapid G12 (KIT3075).

Specificity

This test can specifically detect the presence of the toxic fraction of the prolamins of wheat (gliadin), rye (secalin), barley (hordein) and some varieties of immunogenic oats (avenin) that can therefore be harmful for celiac patients [ref. 2]. However, when the samples contain celiac-safe foods like rice, corn, soy, buckwheat, sesame, millet, teff, quinoa and amaranth, no positive signal is observed.

Internal Validation

To ensure the test's performance with all types of food and other materials such as cosmetics and personal care products, a broad range of commercial products have been tested with GlutenTox Sticks Plus. In all types of matrices tested (see Tables 1 and 2), the results were satisfactory and consistent with the gluten contents determined with the approved method of Codex Alimentarius.

Table 1. Food Samples Tested for Validation of GlutenTox Sticks Plus

Group	Tested samples
Flour and semolina	Corn flour, precooked corn flour, corn semolina, rice flour, wheat flour, buckwheat flour
Milk products	Cow milk, milk with soluble fiber, milk with cereals, flavored or natural yogurt, cheese spread, shredded cheese blend
Baked and cereal products	Toast, bread sticks, biscuits (rich tea), chocolate cookies, Madeleine, cake, cornflakes, pasta, corn pancakes, rice cakes, spelt cake, snacks
Meat products	Minced turkey, minced chicken, turkey sausage, chicken nuggets, pork sausages, chorizo, pork liver pâté
Fishery products	Cod and Hake
Vegetables	Lettuce mix, fried vegetables
Broth, soups, creams and dry mixes	Vegetable broth, chicken rice soup, dehydrated vegetable soup, stock cubes, vegetable soup, peanut butter
Sauces, dressing, spices and condiments	Yogurt salad dressing, ketchup, soy sauce, salad dressing, garlic powder, paprika powder, cooking cream
Sugars	Glucose syrup, powdered sugar
Prepared meals and dishes	Meatballs in sauce with peas, meat ravioli in egg dough, bean stew
Fatty foods	Olive oil, sunflower oil, butter, margarine, cream
Acidic foods	Tomate sauce, wine vinegar, apple cider vinegar, lemon juice
Beverages	Water, milk, fruit juices, beer, soy drinks, rice drinks, oat drinks, soft drinks

Table 2. Non-food Samples Tested for Validation of GlutenTox Sticks Plus

Group	Tested samples
Personal care products	Bath gel, shampoo, deodorant, toothpaste, mouthwash
Cosmetics	Creams (face, body and hands), cleansers, lip balm
Others	Pet food (dry food, wet food), cleaning products, drugs (tablets, capsules and syrups)

14. Intellectual property

The immunoreagents used in this kit are commercialized under the exclusive license for biological material from the Spanish National Research Council (CSIC).

15. References

1. Shan, L., et al., "Structural basis for gluten intolerance in celiac sprue"; *Science*, 2002, 297: 2275-2279.

2. Comino, I., et al.; “Diversity in oat potential immunogenicity: basis for the selection of oat varieties with no toxicity in coeliac disease.”, *Gut*, 2011; 60:915-922.

3. Morón, 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", *AJCN*, 2008; 87:405-414.

4. Síglez, M.A., et al.; "Método de detección de gluten en superficies", *Alimentaria*, 2010; 411:67-70.