



GlutenTox Sticks

Quick test for the detection of gluten in foods

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(Cat. No. KT-4711) For *in vitro* diagnostics use.

Lot.GL007

GLUTENTOX STICKS

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1. Purpose

GLUTENTOX is an immunochromatographic rapid test for the semi-quantitative detection of immunotoxic gluten in foods, harmful for celiac disease patients.

2. Introduction

Celiac disease is a disorder that occurs in people of all ages. Celiac disease results in nutrients from food (proteins, fat, carbohydrates, mineral salts, and vitamins) passing through the stomach without being absorbed (malabsorption), leading to diarrhea, vitamin and mineral deficiencies, anemia and thin bones (osteoporosis). This disease is caused by an inappropriate immune system response to gluten (a mix of proteins present in cereals) from wheat, rye, barley and, to a lesser extent, oat [ref. 1].

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 daily use products such as the adhesives of stamps and envelopes.

The *Codex Alimentarius* Commission considers a food “gluten-free” when its gluten content is less than 20 ppm.

3. Test basis

GlutenTox is a semi-quantitative immunochromatographic test for the detection of gluten in foodstuffs. During the test, the sample reacts with the coloured conjugates (anti-gliadin 33 mer monoclonal antibody –red coloured microsphere) previously fixed into the test [ref. 2]. This complex spreads by capillarity through the membrane of the test. If the result is positive, a red-coloured line appears in the result zone of the membrane. The absence of the line suggests a negative result. Whether or not gluten is present, the mix of the conjugate moves through the membrane up to the control region where antibodies have been set and a BLUE line (control line) will always appear. The appearance of this line is used: 1) to verify that the sufficient volume of sample has been added, 2) that the flow has been appropriate, and 3) as an internal control of the reagents.

4. Storage conditions

To obtain optimal test performance, the product must be stored in its original packaging, between 2 and 30°C and used before expiry date.

Warning: The product should not be opened until its time of use. Very important: always keep the tubes with the tests closed as tightly as possible. Once the seal is broken, keep the tube with the tests at room temperature. To avoid water condensation, do not refrigerate the tubes after opening. Do not freeze.

5. Precautions

- Only for professional *in vitro* use.
- Do not use after expiry date.
- Do not touch the sticks, especially the lower absorbent part, with scrupulously washed hands before having, given that this may result in false positives.
- The use of non-powdered disposable gloves is recommended.

6. Supplied materials

- GlutenTox Sticks (25 sticks)
- Extraction Solution (Ethanol 60%, 250 ml)
- Dilution Solution (30 ml)
- Positive Control (wheat flour, 10 g)
- Negative Control (corn flour, 10 g)
- Instructions for use

7. Necessary materials not supplied

- Analytic scale
- Closing test tubes (>10 ml)
- Test vials
- Pipettes and tips
- Vortex agitator (optional)
- Wheel agitator (optional)
- Centrifuge (optional)
- Disposable gloves
- Distilled water
- Stopwatch

8. Gathering of samples and preparation

Solid samples extraction and controls:

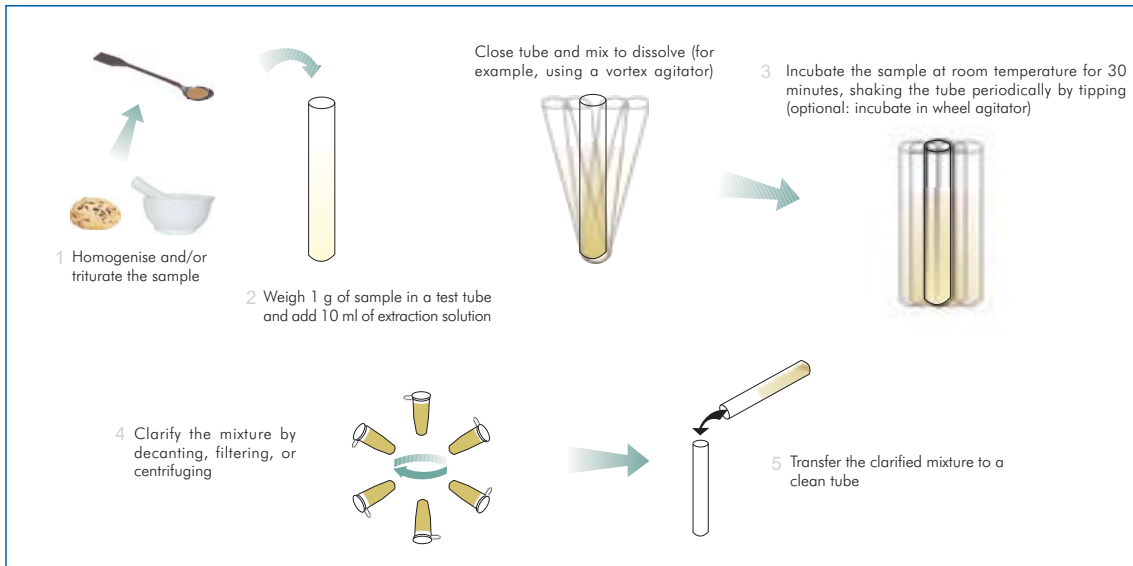
1. Homogenise, mill and/or triturate the sample.
2. Weigh 1 g of sample in a test tube and add 10 ml of extraction solution. Close tube and mix to dissolve (for example, using a vortex agitator). For provided positive and negative controls, perform the same procedure.
3. Incubate the sample at room temperature for 30 minutes, shaking the tube periodically by tipping it over (optional: incubate using a wheel agitator).
4. Clarify the sample to eliminate solids by decanting, filtering, or centrifuging. Solid parts can alter the results.
5. Transfer the clarified mixture to a clean tube.

Liquid samples extraction:

Liquid samples do not require the extraction process and are analysed directly.

! NOTE: Samples, once extracted, must be analysed as quickly as possible.

! NOTE: Some heat processed food may need special extraction solution [ref. 4]



9. Analysis of the samples

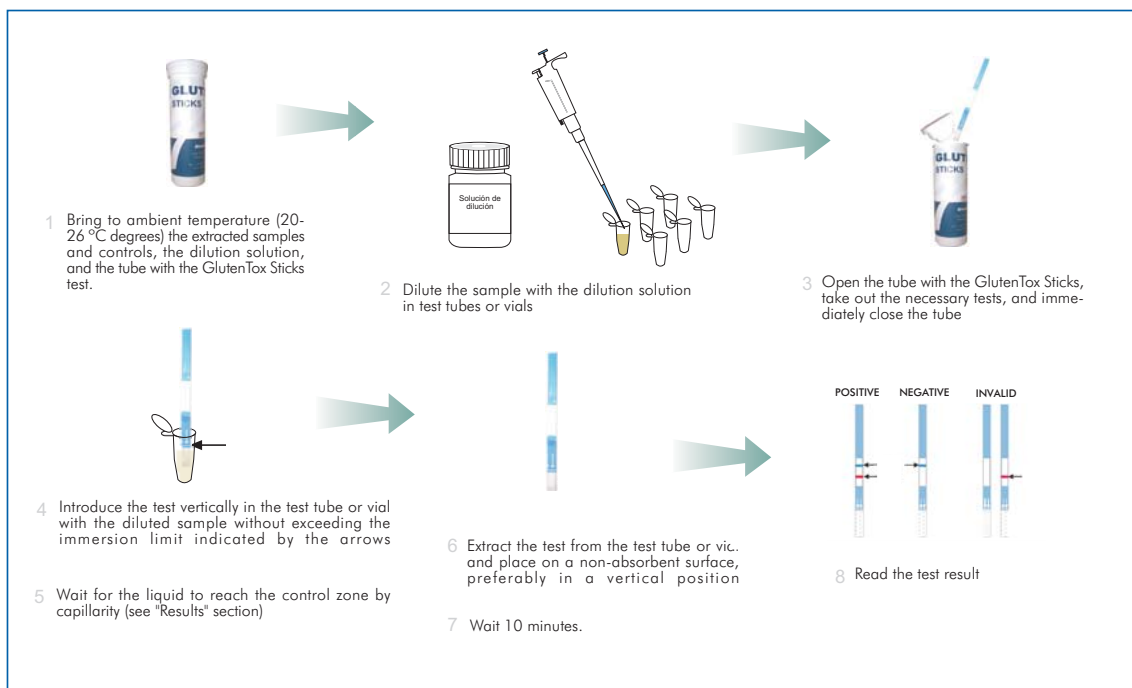
1. Bring the extracted samples, controls, the Dilution Solution, and the tube with the GlutenTox Stick to room temperature (20-26 °C degrees).
2. Dilute the sample with a dilution solution in test tubes or vials, using a maximum volume of 1 ml (ie. for a 1:30 dilution, add 30 µl of extracted sample in 870 µl of dilution solution (see table point 13):



Samples with extraction: dilution 1/10; 1/30; 1/60 or 1:300

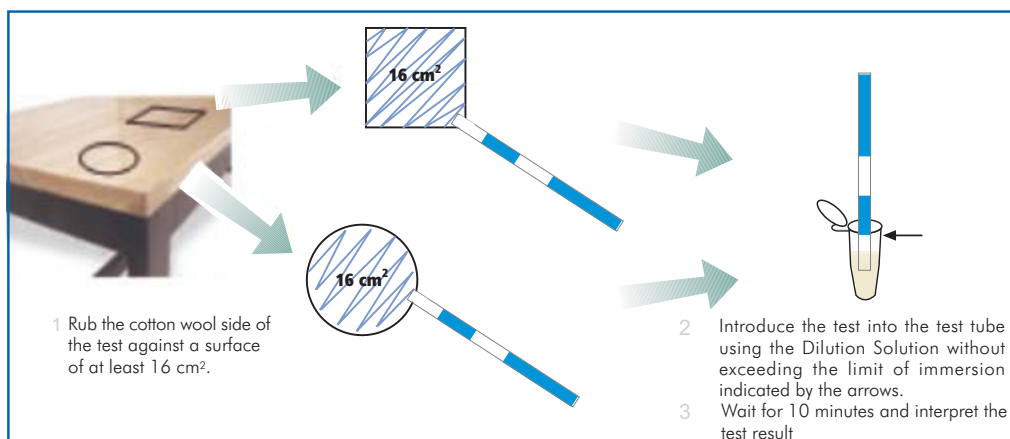
Samples without extraction: dilution 1/100; 1/300; 1/600 or 1/600

3. Open tube with GlutenTox Sticks, extract necessary tests and immediately close tube.
4. Introduce the test vertically in the test tube or vial with the diluted sample without exceeding the immersion limit indicated by the arrows.
5. Wait for the liquid to reach the control zone by capillarity (see "Results" section).
6. Extract the test from the test tube or vial and place on a non-absorbent surface, preferably in a vertical position.
7. Wait 10 minutes.
8. Read the test result.

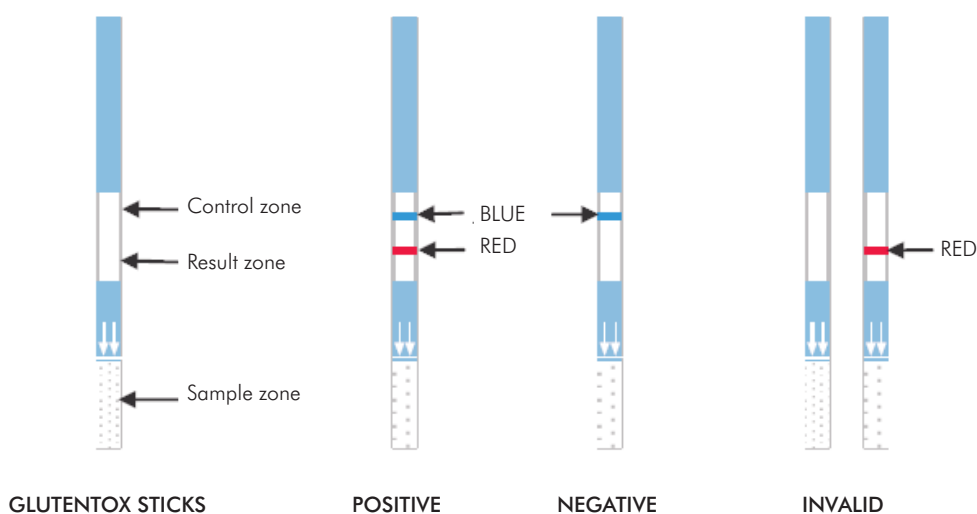


9. 1. Surface Analysis

1. Rub the cotton wool side of the test against a surface of at least 16 cm² (see appendix 1).
2. Introduce the test into the test tube using 100 microlitres of Dilution Solution without exceeding the immersion limit indicated by the arrows.
3. Wait for 10 minutes and interpret the test result.



10. Results



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

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

INVALID: When 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 persists, you must contact the supplier and stop using the test.

! NOTE: The intensity of the red colour line in the results zone will vary depending on the gliadin concentration present in the sample.

11. 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.

12. Limitations

1. Once the package is open and the test taken out, the test must be used immediately.
2. This test is very sensitive, and touching the sticks with the hands must be avoided, especially, the lower absorbent part, given that it may result in false positives.

13. Features of GLUTENTOX

Trials have been undertaken to determine the sensibility of the test using solutions with various concentrations of gliadin in the dilution solution.

Sensitivity

The detection limit of the test is of 15 ng/ml of gliadina.

As regards ppm of gluten, the detection limit of the sticks will depend on the dilution made.

Therefore:

Samples with extraction:

Gluten Detection Limit in ppm				
Dilutions				
	1:10	1:30	1:60	1:300
Positive	> 3 ppm	> 10 ppm	> 20 ppm	> 100 ppm
Negative	< 3 ppm	< 10 ppm	< 20 ppm	< 100 ppm

Samples without extraction:

Gluten Detection Limit in ppm				
Dilutions				
	1:100	1:300	1:600	1:3000
Positive	> 3 ppm	> 10 ppm	> 20 ppm	> 100 ppm
Negative	< 3 ppm	< 10 ppm	< 20 ppm	< 100 ppm

For 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.

Therefore, if the results is positive, a minimum of 10 ng/cm² is detected.








Specificity

GLUTENTOX can specifically detect the presence of the toxic fraction [ref. 1] of the prolamins of **wheat** (gliadin), **rye** (secalin), and **barley** (hordein), toxic for celiac disease sufferers. However, no positive sign is shown when the samples contain rice and corn, cereals that are safe for celiac disease sufferers.

14. References

1. SHAN L., et al.; "Structural basis for gluten intolerance in celiac sprue"; Science; 2002; 297: 2275-9.
2. AWAD A. OSMAN et al.; "Monoclonal antibody recognizing a potential coeliac toxic repetitive pentapeptide epitope in gliadins"; 15th Meeting Working Group on Prolamin analysis and toxicity, Nov 2000, Meran, Italy. pp. 143-146.
3. DENERY-PAPINI S., et al.; "Extraction and immunochemical measurement of raw and heated gluten in food", 15th Meeting Working Group on Prolamin analysis and toxicity, Nov 2000, Meran, Italy. pp. 139-142.
4. GARCÍA E., LLORENTE M., HERNANDO A., KIEFFER R., WIESER H., MÉNDEZ E.; "Development of a general procedure for complete extraction of gliadins for heat processed and unheated foods", Eur J Gastroenterol Hepatol, 2005 May; 17(5):529-39.

15. Symbols for reagents and products for in vitro diagnostics

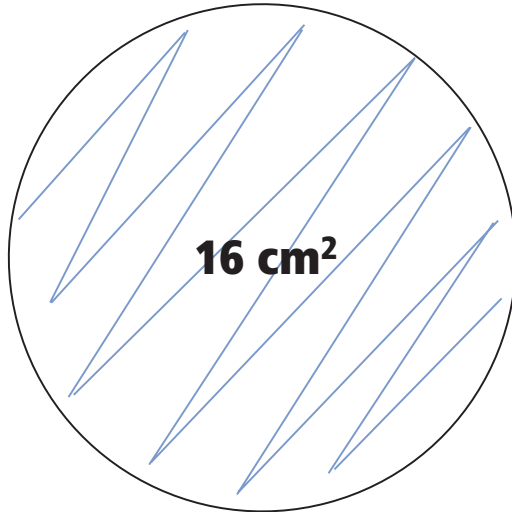
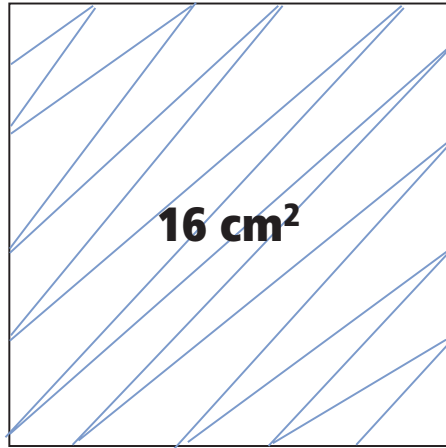
	Consult instructions for use		Lot number
	Store in dry place		Reference number
	Temperature limit		Contains <n> tests
 Expiry date			

16. Related products and/or services

Product/Service	Cat.No.
ELISA Sandwich Analysis Service	SE-4692
ELISA Competitive Analysis Service	SE-4693
GlutenTox Sticks (25 sticks)	RS-4688
Extraction Solution (Ethanol 60%, 250 ml)	RS-4706
Dilution Solution (30 ml)	RS-4707
Positive Control (wheat flour, 10 g)	RS-4708
Negative Control (corn flour, 10 g)	RS-4709
Test tubes (25 units)	RS-4710

Appendix 1

Minimal surface to analyze.



NOTES



For more information,
please visit our WEBSITE
or contact us:

SEVILLA

Av. Americo Vespucio, nº 5-E, pl 1^ª
Parque Científico Tecnológico Cartuja 93.
41092 - Sevilla - España/Spain

Tel. +34-954081276
Fax. +34-954081279

Biomedal S.L.
www.biomedal.com
info@biomedal.com