hygiena™

GlutenTox® Pro

Gluten detection kit for foods, drinks and working surfaces

PERFORMANCE TESTED
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REF KIT3000 (KT-5660)

GlutenTox® Pro

Contents

1. Intended purpose 2
2. Introduction 2
3. Test basis 2
4. Supplied materials (for 25 tests) 3
5. Useful but not supplied materials 3
6. Storage conditions 3
7. Precautions 3
8. Warnings and limitations 3
9. Food 4
    9.1. Preparation and sample analysis 4
    9.2. Interpretation of results 7
10. Surface analysis 7
    10.1. Preparation and sample analysis 7
    10.2. Interpretation of results 8
11. Quality control 8
12. Analytical features 8
    12.1. Sensitivity 8
    12.2. Specificity 9
    12.3. Internal validation 9
13. Intellectual property 9
14. References 9

Appendix 1 10

Appendix 2 11

1. Intended purpose

GlutenTox® Pro is a rapid and user-friendly test for the detection of gluten*, which is harmful for celiac disease sufferers, in food** and on surfaces**. (Food matrices and environmental surfaces approved by AOAC are listed in Appendix 1). This kit is recommended for consumers, commercial kitchens and industry and includes the possibility of choosing different detection threshold levels of gluten according to the end user requirements.

* not for hydrolyzed sources of gluten.
** Matrices validated according to AOAC Performance Tested Methods (PTM) protocols:
- Food matrices: Rice flour, bread, rolled oat, pâté and yogurt.
- Environmental surfaces: stainless steel, rubber, plastic, food-grade painted wood and sealed ceramic.

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 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® Pro is an immunochromatographic test (lateral flow test) for the detection of gluten in foods with different composition and levels of processing, from raw materials to processed food. In addition, GlutenTox® Pro can be used to control the cleanliness of food production zones through surface analysis, a prerequisite to prevent the risk of cross-contamination in the final product.

The test consists of an extraction stage using a simple procedure which is common to all types of food. The detection step is based on the reaction of the 33mer-like immunotoxic peptides of gluten in the sample with the colored conjugates (monoclonal anti-gliadin 33mer antibody / red colored microsphere) previously fixed on the stick [ref. 3 and 4]. This complex spreads by capillarity through the stick. 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 mixture of the conjugate moves through the stick up to the control region where, if the test was properly performed, a BLUE line (control line) will appear, due to accumulation of blue microspheres included also in the stick.

These rapid tests are specially 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. They also enable quick decisions and corrective actions in case there is any risk of contamination along the production chain.

4. Supplied materials (for 25 tests)

- GlutenTox® Pro stick (x25) in a tube.
- Plastic pipette (x50).
- Disposable plastic spoons (x25).
- Yellow cap bottle with extraction solution (x25).
- Blue cap bottle with dilution solution (x25).
- Instructions leaflet.

5. Useful but not supplied materials

- Mortar or any other utensil to grind the sample.
- Non-powdered disposable gloves.
- Scale (precision 0.1 g).
- Alcohol (ethanol).
- Watch (a stopwatch is preferable).

6. Storage conditions

The product must be stored at a temperature ranging from 15 – 25 °C (59 - 77 °F) during the shelf life of the kit. To obtain optimal test performance, the product must be stored in its original packaging, and used before the expiration date printed on the tube of sticks.

WARNING: The tube with the sticks should not be opened until its time of use. All components of the kit are fully disposable in ordinary trash or recyclable where appropriate.

7. Precautions

- To avoid contaminations that interfere with the analysis, the use of non-powdered disposable gloves is recommended. If you do not have disposable gloves, wash your hands thoroughly before the test.
- Once the GlutenTox® Pro stick has been removed from the tube, it must be used as soon as possible under strict clean conditions. Close the tube afterwards.
- Do not use any material from the kit after the expiration date.
- Do not drink any solution (liquid) from the kit (the extraction solution contains alcohol [ethanol].
- **Keep out of reach of children.**

8. Warning and limitations

- In certain types of samples gluten may be extracted with lower efficiency, and therefore working under conditions of maximum sensitivity (10 drops of the extract to the dilution bottle with blue cap) is recommended to ensure a detection limit of at least 10 ppm. These samples are the following:

  - Food containing ingredients (up to 70%) having high concentrations of polyphenols or tannins (e.g. chocolate, black tea, coffee, wine, berries, legumes like chickpeas or lentils, etc.)
  - Samples with antioxidants, such as vitamins A, E and C.
  - Food heat-treated with temperatures above 180 °C/356 °F.

- In extensively hydrolyzed matrices such as beer, syrups or sourdough, the gluten content of the sample could be underestimated.

IMPORTANT NOTE!

- **In these cases it has been found that at least 50% of the total content of gluten present in the sample is detected. Therefore, if a negative result is obtained by working under conditions of maximum sensitivity (with 10 drops), it can be assured that the food contains less than 10 ppm of gluten, and therefore, it can be considered as "gluten-free" suitable for celiacs in accordance with current regulations.**

- In some food samples with a very high content of polyphenols and tannins, i.e. foods in which the major component (> 70%) is chocolate, black tea, coffee, wine, berries, legumes like chickpeas or lentils, etc., the extraction process can be insufficient and therefore underestimate the amount of gluten in the sample. In these cases it is recommended to use GlutenTox® Sticks Plus*.

- Gum-type samples can be difficult to analyze due to the thick paste formed when mixed with the extraction solution provided in the GlutenTox® Pro Test Kit. Please ask your kit provider for advice.

*For more information contact your supplier.

9. Food

9.1. Preparation and sample analysis

IMPORTANT NOTE!

- **Before using the kit clean the utensils and the areas in which the sample will enter in contact, with soap and water, and rinse well. After cleaning, it is highly recommended to wipe them with a clean cloth dampened with alcohol. Materials included in the kit are ready to use, and can be disposed after use.**

9.1.1. If the sample is liquid, shake it vigorously to homogenize completely before sampling.
If the sample is semi-liquid or doughy, remove it with the help of a tooth-pick or clean spoon to mix it and achieve a homogeneous mixture.
If the sample is solid, grind it as much as possible using a mortar and/or a domestic meat grinder/mincer which must be perfectly clean. If the food sample is too hard (sweets, nougat, etc.) fragment it with a knife or a little hammer to achieve an efficient grinding.

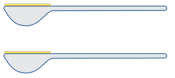
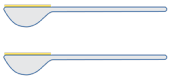
IMPORTANT NOTE!

- **If a food has several parts, be sure to take a representative sample of each and prepare a final homogeneous sample. If you do not do this, and the gluten was distributed unevenly in the food, a false negative could be obtained.**

9.1.2. Use the provided spoon with leveled amounts of sample following Table 1 or if you have an appropriate scale[1] weigh 1 gram of sample. If the sample is liquid, use one leveled spoon or take 1 milliliter of the liquid.

[1] The use of a scale increases the accuracy of the analysis process.

Table 1. Amount of sample depending on the kind of food

Type of food	Examples	Spoonful
Flours, fine powders	Corn flour, rice flour*, milk powder, spices, rolled oat*	
Fine crumbs	Bread*, cookies, cakes, snacks, etc.	
Liquids and sauces	Water, rinse water, milk, juice, condensed milk, yogurt*, soup, gravy, sauce, cream, etc.	
Meat, fish and cold meat	Meat, fish, sausage, black pudding, pâté*, canned meat and fish, etc.	

* Food matrices approved by AOAC.

9.1.3. Open one extraction bottle with yellow cap. Add the content of the spoon, 1 gram or the milliliter to the extraction bottle with yellow cap.

9.1.4. Close the bottle, shake vigorously for at least 2 minutes and let it settle for about 5 minutes so that any solids rest on the bottom of the tube. Settling time will depend on the type of sample.

9.1.5. Open one dilution bottle with blue cap. Using a disposable plastic pipette, take approximately 1 mL of extracted sample from the extraction bottle with the yellow cap. Add 10, 4, 2, or 1 drops of extracted solution to the dilution bottle with the blue cap according to your required threshold/limit of detection (see Table 2).

Mix softly for at least 15 seconds.

Table 2.

no. drops	LD
10	5 ppm
4	10 ppm
2	20 ppm
1	40 ppm

9.1.6. Using a new disposable plastic pipette, add 10 drops from the dilution bottle with blue cap in the same inverted blue cap. Put the cotton wool side of the stick in contact with the liquid present in the blue cap until all the liquid is absorbed. Let it stand in the blue cap.

9.1.7. **Wait 10 minutes** to see the final result (if there is a high concentration of gluten, the result may appear in less than 1-2 minutes).

IMPORTANT NOTE!

- **Wait 10 minutes to read the result. Do not leave the stick longer than indicated, as the results may vary.**

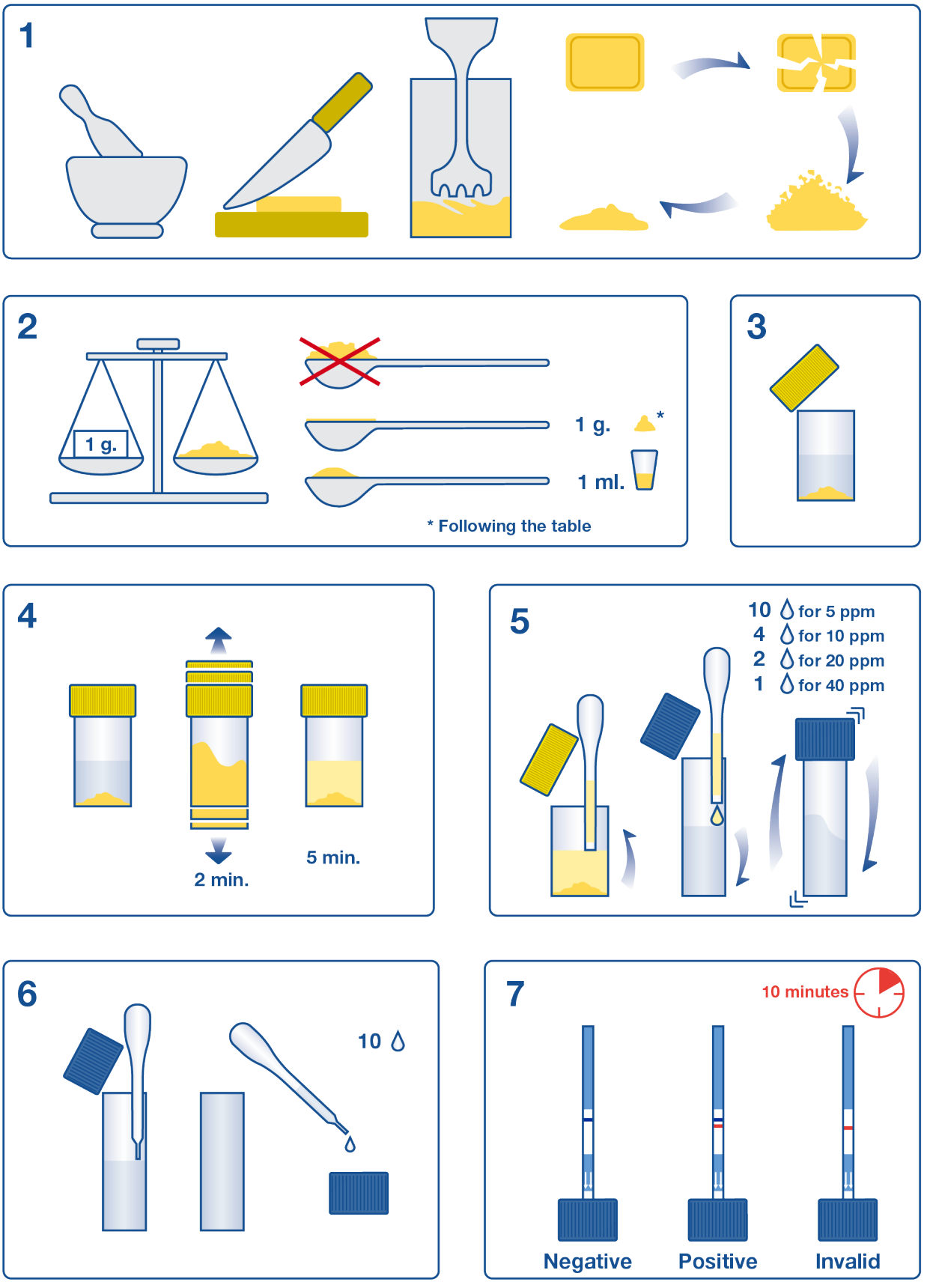


Figure 1. Preparation and analysis of samples.

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

The threshold of detection depends on the number of drops (10, 4, 2 or 1 drops) added to the dilution bottle with blue cap in step 9.1.5. (see Table 3).

Table 3. Interpretation of results [2]

		Detection threshold			
		10 drops	4 drops	2 drops	1 drop
Test Result	Positive	> 5 ppm	> 10 ppm	> 20 ppm	> 40 ppm
	Negative	< 5 ppm	< 10 ppm	< 20 ppm	< 40 ppm

[2] The results are qualitative for limit of detection.

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 repeat the experiment with a new test always following a correct procedure. If the problem persists, you must contact the supplier and stop using the test.

The test is for screening purposes. A positive result might require confirmation or further testing.

10. Surface analysis

10.1. Preparation and sample analysis

10.1.1. Rub the cotton wool side of the stick against a surface of at least 16 cm^2/2.46 $inch^2$ or in a line of 40 cm/15.6 inch. The area selected for analysis must be representative of the total area of interest.

10.1.2. Open a dilution bottle with blue cap and place inverted cap on a clean surface. Use a new, disposable plastic pipette to place 10 drops from the dilution bottle into the blue cap. Put the cotton wool side of the stick in contact with the liquid present in the blue cap until all the liquid is absorbed. Let it stand in the blue cap.

10.1.3. **Wait 10 minutes** to see the final result (if there is a high concentration of gluten, the result may appear in less than 1-2 minutes).

NOTE!: Environmental surfaces approved by AOAC are listed in Appendix 1.

IMPORTANT NOTE!

- **Wait 10 minutes to read the result. Do not leave the stick longer than indicated, as the results may vary.**

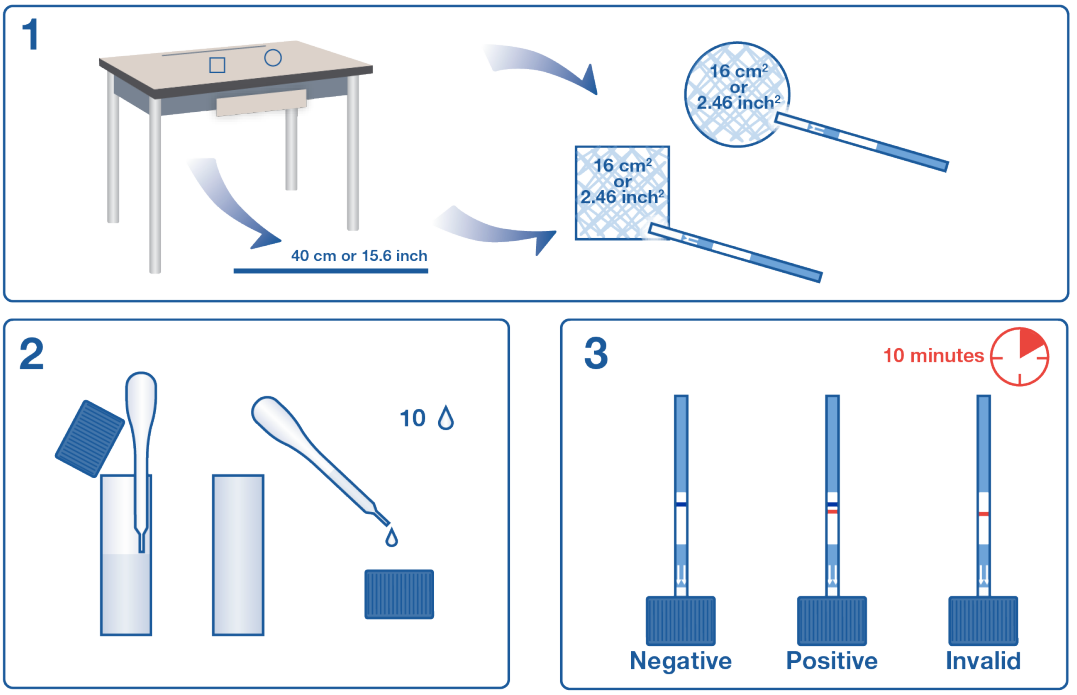


Figure 2. Procedure for surface analysis.

10.2. 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.

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 same considerations apply as for paragraph 9.2 invalid test results.

11. Quality control

The internal control is included in the test. The blue line that appears on the stick is the internal control of the test which checks that the sample volume is sufficient and that the followed procedure is adequate.

12. Analytical features

12.1. Sensitivity

Food

The limit of detection of the assay is 5 ppm, working at maximum sensitivity (10 drops). This detection limit is always reached in simple samples, such as raw materials, foods with little degree of processing and non heat-processed foods. The high sensitivity of the test complies fully with the Codex Alimentarius guidelines and Regulation (EC) 41/2009, which sets a limit of 20 ppm to consider the food as "gluten-free".

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.

Positive results from a surface size of 16 cm^2/2.46 $inch^2$ or a line of 40cm/15.6 inches indicate a minimum detection of 10 ng/cm^2 of gluten from the surface [ref. 5].

12.2. Specificity

This test can specifically detect the presence of the toxic fraction (33mer) of the prolamins of wheat (gliadin), rye (secalin), barley (hordein) and as well varieties of oat [ref.2] (avenin) that can be toxic and therefore harmful for celiac patients. Furthermore, the test will not cross-react with samples containing rice, corn, soy, buckwheat, sesame, millet, teff, quinoa and amaranth.

Note: To estimate gluten in a sample with this test, the prolamin content has been adjusted multiplying by factor = 2.

12.3. Internal validation

In addition to the food matrices and environmental surfaces validated according to AOAC Performance Tested Methods (PTM) protocols, listed in Appendix 1, to ensure the test's ability to analyze a wide range of samples of different types (food, beverages, oral hygiene products), different commercial samples have been tested. After analyzing the samples with GlutenTox® Pro in all types of matrices (see Appendix 2) the results were satisfying and consistent with the validated method for the Codex Alimentarius, which demonstrates that the test can be used on a broad range of samples.

13. Intellectual property

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

14. References

1. SHAN L., et al.; "Structural basis for gluten intolerance in celiac sprue"; Science; 2002; 297: 2275-9.

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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", Am. J. Clin. Nutr, 2008; 87:405-414.

4. MORÓN B., et al.; "Toward the Assessment of Food Toxicity for Celiac Patients: Characterization of Monoclonal Antibodies to a Main Immunogenic Gluten Peptide" PLoS ONE 2008; 3(5): e2294.

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

Appendix 1. Matrices validated according to AOAC Performance Tested Methods (PTM) protocols

Group	Tested samples
Foods	Rice flour, bread, rolled oat, pâté and yogurt
Environmental surfaces	Stainless steel, rubber, plastic, food-grade painted wood and sealed ceramic.

Appendix 2. Samples tested for internal validation of GlutenTox® Pro by Hygiena

Group	Tested samples
Flour and semolina	Corn flour, precooked corn flour, corn semolina, rice flour*, buckwheat flour, rolled oat*
Milk products	Cow milk, milk with soluble fiber, milk with cereals, natural or flavored yogurt*, cheese spread, shredded cheese blend
Baked and cereal products	Toast, bread stick*, biscuits (rich tea), chocolate cookies, Madeleine, cake, cornflakes, pastas, corn pancakes, rice cakes, spelt cake, snacks
Meat products	Minced turkey, minced chicken, turkey sausage, pâté*, chicken nuggets, pork sausages, chorizo
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	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	Tomato sauce, wine vinegar, apple cider vinegar, lemon juice
Beverages	Water, rinse water, milk, fruit juices, soy drinks, rice drinks, oat drinks, soft drinks
Oral hygiene products	Toothpaste, mouthwash

* Food matrices approved by AOAC.

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Notes

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