Micro biologique

# OnSite® Gluten Test Kit

## Instructions For Use

Detection test for gluten in foods and environmental surfaces

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 012501

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## 1. Introduction

Wheat, barley, and rye contain a form of gluten that can trigger symptoms of Celiac Disease and other gluten-related disorders in susceptible people. Disease management involves avoidance of gluten consumption. However, many gluten-free (GF) alternatives are frequently contaminated with gluten. To help protect from inadvertent exposures, regulatory bodies have adopted standards that limit gluten contamination levels in GF foods. Compliance with these standards is achieved through the application of antibody-based assays that can detect gluten contamination at or below this established value. To assist the food industry in establishing effective food safety practices, Microbiologique has developed the OnSite® Gluten Test Kit to detect gluten in select food products and on surfaces in less than 20 minutes.

## 2. Intended Use

The OnSite® Gluten Test is designed to detect gluten from wheat, barley, and rye present in baked foods and wheat gluten in raw foods as well as on environmental surfaces. The LOD for environmental surfaces is 11 µg/100 $cm^2$ gluten and the LOD for select foods can be variably adjusted to 5, 10, or 20 mg/kg (ppm) gluten, depending on the matrix. The kit is not suitable for the detection of hydrolyzed and/or fermented gluten. For information on potential limitations and troubleshooting, see Sections 9 and 10.

The assay is intended for laboratory and industry use, including within food production facilities, commercial kitchens, contract laboratories, and auditing programs. Testing should only be performed by trained personnel.

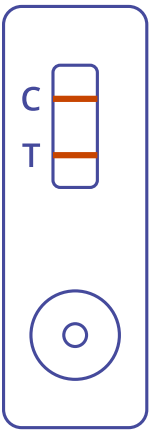
**Please read ALL instructions prior to use.**

## 3. Performance Characteristics

**Limit of Detection**: For environmental surfaces, the limit of detection is 11 µg/100 $cm^2$ gluten. For foods, the LOD can be variably adjusted to 5 10, or 20 mg/kg (ppm) gluten depending on the matrix.
**Operation Time:** 15-20 min
**Suitability:** Non-hydrolyzed gluten residues, both native and chemically modified (deamidated).

## 4. Assay Principles

The OnSite® Gluten Test is a lateral flow assay that rapidly detects gluten derived from wheat, barley, and rye in select foods as well as on environmental surfaces. The kit is based on the monoclonal antibody 2D4 configured in sandwich format. 2D4 replicates the characteristics of the R5 antibody while additionally detecting deamidated gluten as well[1]. To operate the kit, the sample is briefly extracted, diluted in a running buffer, and then directly applied to the device. The fluid is then wicked across the reagent zone, which includes a procedural control line (C) and a test line (T). The test outcome is interpreted by visualizing the appearance of these lines at 10 min.




## 5. Kit Storage and Safe Handling

- ★ Store in original packaging at 2-30 °C (36-86 °F). Refrigeration (2-8 °C) is suggested for long-term storage. DO NOT FREEZE.
- ★ Do not use kit after printed expiration date.
- ★ Avoid exposure of any components to direct sunlight or heat.
- ★ Dispose of kit components in regular trash, or recycle where appropriate.
- ★ Do not eat or drink any kit components. See SDS for additional information.

[1] Journal of Cereal Science, Volume 108, November 2022, 103585. https://doi.org/10.1016/j.jcs.2022.103585

# 6. Kit Components

- A OnSite® Gluten Test Lateral Flow Devices (x25)
- B Single-use sampling spoons (x25)
- C Single-use droppers (x50)
- D Individually-wrapped sampling swabs (x25)
- E Blue-capped vials with OnSite® Gluten Dilution Buffer (x25)
- F White-capped vials with OnSite® Gluten Extraction Buffer (x25)
- G Instructions for use

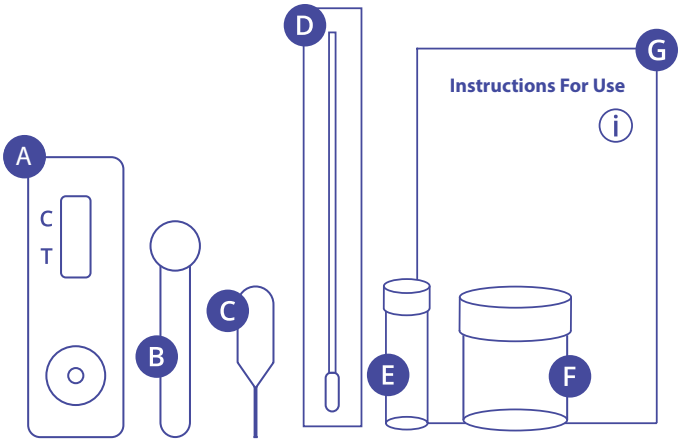



Figure 1. Kit Components

## 6.1. Optional Materials

- Blender, grinder, or similar device for homogenizing sample
- Laboratory timer
- Digital scale sensitive to 0.1g for measuring solid samples
- Calibrated laboratory pipette for measuring liquid samples
- Fine-tipped marking-pen
- Lateral flow device reader
- Non-powdered disposable gloves

# 7. Food Sampling and Analysis

**IMPORTANT: Baked samples should be analyzed via the protocol indicated by this symbol:**

## 7.1. Sample Preparation and Analysis

**7.1.1.** Prior to beginning, ensure ALL test kit components have been brought to room temperature. Thoroughly clean hands, the work area, and requisite utensils to reduce the risk of contamination. Disposable gloves may be used. To adequately perform this task, use detergent and water followed by thorough rinsing. A secondary cleaning with alcohol is strongly recommended.

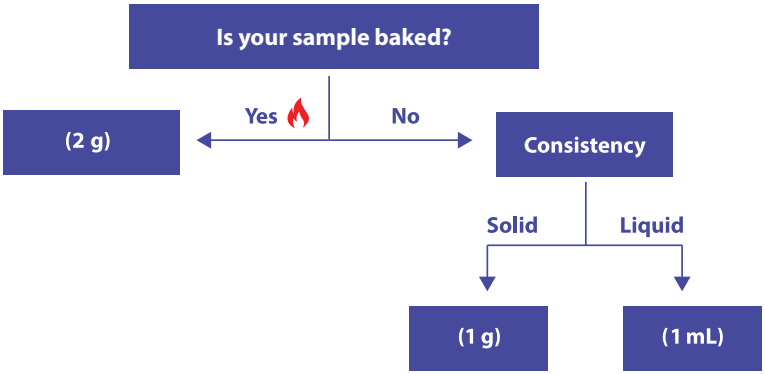
**7.1.2.** A representative sample must be collected from food products. To ensure an accurate analysis of the final product, make sure that all components of the final product are present in the sample.

**7.1.3.** To ensure consistent results, a suitable blender, food processor, or a similar mixing device should be used to homogenize **solid samples**. **Liquid samples** should be mixed or shaken vigorously before sampling. Samples of a doughy consistency, or high in viscosity can be mixed using clean metal utensils.

**7.1.4.** Collect the appropriate amount of sample as indicated in **Figure 2.** For baked foods, collect 2 g of solid sample. For other foods, collect 1 g of solid sample or 1 mL of liquid sample.

A precise sample size is critical for accurate results. Use of a weigh balance or calibrated pipette is recommended, however the enclosed spoons may also be used.

Figure 2. Required Sample Amount Based on Sample Composition.




**7.1.5.** Open one **white-capped extraction vial** and add the measured sample into the vial.

**7.1.6.** Replace the lid tightly. Shake vial vigorously for two minutes.

**7.1.7.** Allow sample to settle for 5 minutes.

**7.1.8.** Open one **blue-capped dilution vial** and set to the side. Open the white-capped extraction vial and withdraw a portion of the top extraction solution using one of the provided droppers.

**Note: Avoid withdrawing any of the sample particles that have settled to the bottom of the tube and avoid withdrawing foam that may be present on the surface.**

**7.1.9.** Add the number of drops of extract that correspond to the desired detection level to the blue-capped dilution vial **(see Table 1).** Replace the cap firmly, then gently mix by inversion for 15-20 seconds.

Table 1. Drops of Extracted Sample per Detection Level

| Detection Level | No. of Drops | Detection Threshold |
|---|---|---|
| Low | 8 | 5 ppm |
| Medium | 4 | 10 ppm |
| High | 2 | 20 ppm |

**NOTE: For rye gluten incurred bread, the detection threshold is 5 ppm at all detection levels**

**7.1.10.** Open and remove a new lateral flow test device and set it onto a flat, level surface. Using a new provided dropper, transfer **6 drops** of the diluted sample from the **blue-capped dilution vial** to the sample port on the lateral flow test device. Alternatively, if using a calibrated pipette, transfer 100 µL to the sample port.

**7.1.11.** Wait exactly 10 minutes before interpreting the test result.

**NOTE: Samples containing high levels of gluten may produce an accurate, positive test result more quickly. However, a negative result can only be declared after the full 10 minutes. To ensure accuracy and to avoid misinterpretation of drying artifacts, analyze results promptly at 10 minutes.**

## 7.2. Interpreting Test Results

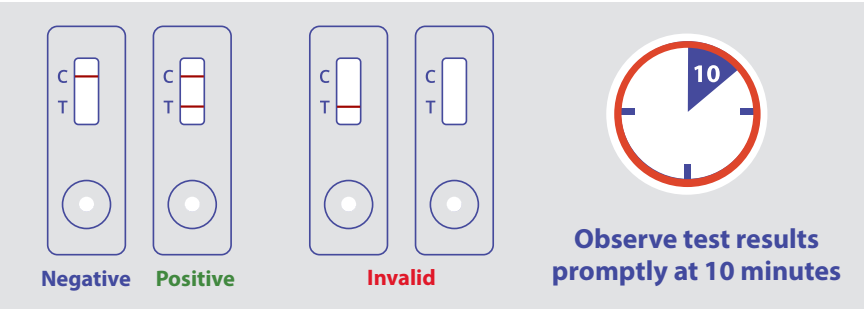



Figure 3. Test Outcomes

**7.2.1.** A negative result is indicated by the development of a strong control line only (upper line "C"). A negative result will not develop color at the test line (lower line). An example of a negative result is displayed in **Figure 3** labeled "**Negative**".

**7.2.2.** A positive result will develop at the test line (lower line "T") in addition to the control line (upper line "C"). The strength of the test line development will be dependent on the amount of gluten present in the sample; any visible line is considered to be a positive result. A positive result indicates that the sample contains at least as much gluten as the selected detection level according to **Table 1**. An example of a positive result is displayed in **Figure 3** labeled "**Positive**".

**7.2.3.** Failure of the control line to appear regardless of test line development is an invalid result. In addition, any line malformation visible on the membrane denotes an invalid test. Examples of malformations would be dark spots, gaps or incomplete line development. Examples of invalid results are displayed in **Figure 3** labeled "**Invalid**". In the event of an invalid test, the procedure should be repeated using a new test device.

# 8. Environmental Sampling and Analysis

## 8.1. Sample Preparation and Analysis

**8.1.1.** Prior to beginning, ensure ALL test kit components have been brought to room temperature. Thoroughly clean hands to reduce the risk of gluten contamination. Disposable gloves may be used. Open a new sampling swab and a new **white-capped extraction vial**.

**8.1.2.** Use a new provided dropper to moisten the swab tip with **8 drops** of extraction solution from the white-capped vial. Close the vial and set it to the side.

**8.1.3.** Collect a surface sample by swabbing a 4 x 4 inch (or 10 x 10 cm) area using a rolling crosshatch technique shown in **Figure 4**. Ensure that all sides of the swab bulb come into contact with the surface.

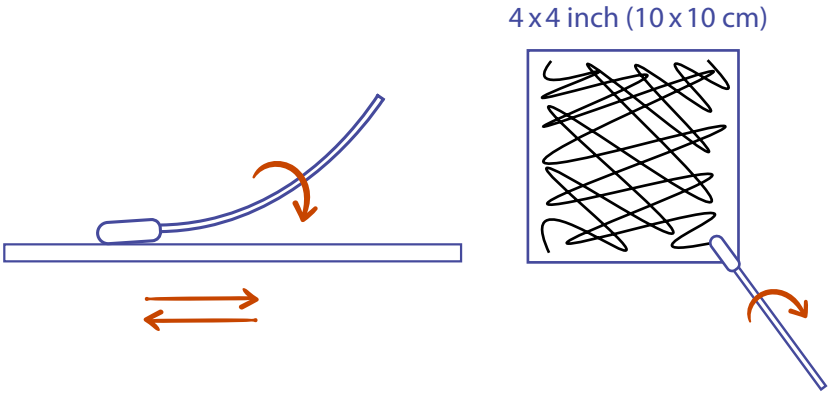



Figure 4. Environmental Surface Sampling

**8.1.4.** Re-open the **white-capped extraction vial** and place the swab tip into the extraction solution. Carefully break the swab stem leaving the tip in the extraction container.

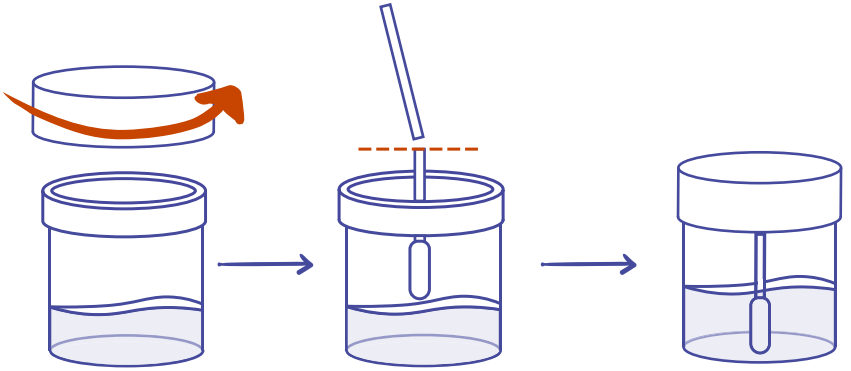


Figure 5. Environmental Sample Extraction

**8.1.5.** Replace the lid tightly. Shake vial vigorously for two minutes.

**8.1.6.** Open one **blue-capped dilution vial** and set it to the side. Using the same dropper as in step 8.1.2, withdraw solution from the **white-capped extraction vial**.

**8.1.7.** Add **15 drops** of the extract to the blue-capped dilution vial. Replace the cap firmly, then gently mix by inversion for 15-20 seconds.

**8.1.9.** Open and remove a new lateral flow test device and set it onto a flat, level surface. Using a new plastic dropper, transfer **6 drops** of the diluted sample from the **blue-capped dilution vial** to the sample port on the lateral flow test device. Alternatively, if using a calibrated pipette, transfer 100 µL to the sample port.

**8.1.9.** Observe test results promptly at 10 minutes.

**NOTE: Samples containing high levels of gluten may produce an accurate, positive test result more quickly. However, a negative result can only be declared after the full 10 minutes. To ensure accuracy and to avoid misinterpretation of drying artifacts, analyze results promptly at 10 minutes.**

## 8.2. Interpreting Test Results

**8.2.1.** A negative result is indicated by the development of a strong control line only (upper line). Negative results will not develop at the test line (lower line). An example of a negative result is displayed in **Figure 3** labeled "**Negative**".

**8.2.2.** A positive result will develop at the test line (lower line) in addition to the control line (upper line). The strength of the test line development will be dependent on the amount of gluten present in the sample. An example of a positive result is displayed in **Figure 3** labeled "**Positive**".

**8.2.3.** Failure of the control line to appear regardless of test line development is an invalid result. In addition, any line malformation visible on the membrane denotes an invalid test. Examples of malformations would be dark spots, gaps or incomplete line development. Examples of invalid results are displayed in **Figure 3** labeled "**Invalid**". In the event of an invalid test, the procedure should be repeated using a new test device.

# 9. Test Limitations and Validated Matrices

**IMPORTANT: Not all samples are suitable for use with this product.**

As with all test kits that rely on antibody-based detection methods, there are additives, matrices, and processing methods that may limit the ability to detect the target analyte. Contact your distributor for technical support regarding sample suitability or help in validating samples for testing.

The OnSite® Gluten Test is designed to detect native and chemically modified (deamidated) gluten. The test may underestimate gluten in matrices containing hydrolyzed gluten, for example gluten-reduced beer.

To maintain accuracy for samples that are baked, in which the gluten may be baked into the finished product, follow the guidance indicated by the .

When testing samples for which baking is uncertain, it is advisable to assume that the sample is baked and extract using 2 g or 2 mL of sample.

The test may underestimate gluten in matrices that have extensive polyphenolic compound content (e.g. chocolate syrup) or those having undergone extensive chemical or thermal processing. Furthermore, foods that are sticky and/or starchy may require additional dilution measures to improve operation. Note that dilution will affect the detection limit of the test.

Matrices validated according to **AOAC Performance Tested Methods** (PTM) protocols: Stainless steel surface, spice mix, rice flour, oat flour, baked bread.

## 10. Best Practices and Troubleshooting

- **Timing is extremely critical.** When testing multiple samples, consider the amount of time required to process each sample. Variation in timing, at different stages of the testing procedure can produce varied results. Once a sample has been extracted, the protocol must be run to completion. Keep in mind that LFD strips should be interpreted at 10 minutes.
- To ensure correct volume of extract, hold the dropper horizontally, parallel to the lateral flow device.
- Do not remove LFD cassettes from foil pouch until the indicated time. Excess exposure to humidity or moisture may cause decreased performance or failure of the test strip.
- Prior to testing, ensure that all components are brought to room temperature.
- When testing highly acidic or basic samples, pH confirmation is suggested. The pH of the diluted sample should be between 6.8 and 7.4. If the pH is not in range, adjust accordingly.
- All tolerances for this assay are temperature +/-5°C, volumes and weights +/-1%.
- Avoid using powdered gloves as this may introduce unwanted gluten.
- Do not re-use kit components.
- Store kit components as indicated.
- Do not use expired reagents.
- Do not mix kit components with other kits or other lot numbers.
- Read test under good lighting.

## 11. Warnings and Customer Support

For Laboratory use only, not intended for human diagnostic use. Testing results are only applicable to the portion of the sample product tested and to this extent, Microbiologique cannot guarantee that gluten is, or is not present in the untested portions of the sample product. Strict adherence to the assay protocol is mandatory to ensure proper operation of the test kit.

All waste must be disposed of in compliance with federal, state, and local rules and regulations. SDS information can be obtained from your local distributor or by emailing: orders@onsitefoodsafety.com.

For additional information on using this kit, please call **866-256-1804** or email **info@onsitefoodsafety.com**.

Additional product information is available at **www.onsitefoodsafety.com**

Microbiologique, Inc.
Address: 8315 Lake City Way NE, Seattle, WA 98115
Website: https://microbiologique.com/gluten-lateral-flow/
Email contact: xf@microbiologique.com or tech@microbiologique.com

# 12. Validated Matrices

The OnSite® Gluten Test kit has been validated as an AOAC Performance Tested Methods℠ (Certificate No. 012501) for the qualitative detection of wheat gluten present in rice flour, oat flour, spice mix, baked bread and on stainless steel surfaces, as well as barley and rye present in baked bread.

**Table 2. AOAC PTM Validated Detection Levels for Gluten in Different Food Matrices**

| Matrix | Test Portion | Gluten Detection Level with $POD_{95}$ [a] | | |
|---|---|---|---|---|
| | | 5 mg/kg | 10 mg/kg | 20 mg/kg |
| Rice flour | 1g | 5 mg/kg | 10 mg/kg | 20 mg/kg |
| Oat flour | 1g | 5 mg/kg | 10 mg/kg | 20 mg/kg |
| Spice mix | 1g | 5 mg/kg | 10 mg/kg | 20 mg/kg |
| Bread with incurred wheat | 2g | 5 mg/kg | 10 mg/kg | 20 mg/kg |
| Bread with incurred barley | 2g | 5 mg/kg | 10 mg/kg | 20 mg/kg |
| Bread with incurred rye | 2g | 5 mg/kg | 5 mg/kg | 5 mg/kg |

[a]Identical results observed in the method developer study and the Independent Laboratory study.

**Table 2. AOAC PTM Validated Detection Levels for Gluten in Environmental Surface**

| Matrix | Gluten Source | Surface Size | Gluten Detection Level with $POD_{95}$ |
|---|---|---|---|
| | | | 11µg/cm² |
| Stainless steel (Swabs) | Wheat flour | 10 x 10 cm | 1.00[a] (0.84, 1.00) |
| | | | 0.95[b] (0.760, 1.00) |

[a]Results from the Method Developer Laboratory study.

[b]Results from the Independent Laboratory study.

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