

The logo for OnSite Allergen Multiplex Tree Nut, featuring three yellow dots of increasing size to the left of the text 'OnSite® Allergen Multiplex Tree Nut' in a bold, blue, sans-serif font.

OnSite® Allergen
Multiplex Tree Nut


Instructions For Use

Rapid detection of tree nuts including almond, cashew, hazelnut,
pecan, pistachio and walnut.

Suitable for rinsewater and environmental surfaces.



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1. Introduction

Food allergies are potentially life-threatening immune-mediated reactions to consuming foods, most notably those containing milk, egg, fish, shellfish, tree nut, sesame, mustard, peanut, wheat, or soybean residues. Roughly 2% of adults and 4-8% of children are affected by food allergies. Management of food allergies requires strict dietary avoidance. To minimize the risk of inadvertent exposures to food allergens, numerous regulatory bodies have adopted strict food labeling requirements. Compliance with these requirements is achieved through the use of antibody-based assays that can detect allergen residues in foods at very low levels. To assist the food industry in establishing effective food safety practices, Microbiologique has developed the OnSite® Allergen Multiplex Tree Nut test kit.

2. Intended Use

OnSite® Allergen Multiplex Tree Nut is a rapid, qualitative test designed to detect tree nut protein in rinsewater and on surfaces. The test can simultaneously detect almond, cashew, hazelnut, pecan, pistachio and walnut residues. To a lesser degree, the test can also detect coconut residues. Results obtained using these tests can be used as part of an effective Allergen Control Plan.

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

Please read ALL instructions prior to use.



3. Performance Characteristics

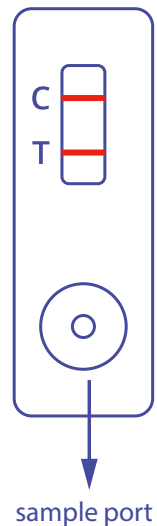
Limit of Detection: between 1-5 mg/kg (ppm) tree nut protein in rinsewater and 10µg/ 100 cm² on surfaces.

Operation Time: 10-20 min

Suitability: Raw and processed tree nut proteins, though detection of extensively hydrolyzed or processed allergens may be reduced. Not intended for high-analyte concentrations; signal will be faint but remain evident at 1,000 mg/kg (ppm) protein in rinsewater. For contamination levels above 1,000 mg/kg (ppm) in rinse water, false negative outcomes may occur.

4. Assay Principles

OnSite® Allergen Multiplex Tree Nut is a test kit that includes lateral flow devices (LFDs) that detect specific food allergens in rinsewater and on environmental surfaces. The kits rely on the use of polyclonal antibodies (pAbs) directed against the target allergens. The test is configured with a test line (T) and a procedural control line (C). The test line (T) will produce a red line at low to medium levels of contamination, whether that contamination is from one or several of the target proteins. The procedural Control Line (C) should always produce a red line, regardless of the analyte concentration. Failure for this line to appear indicates a failure of the mixture to migrate across the membrane. To operate the kit, a rinsewater sample or surface swab is first extracted for 2 min with shaking. Thereafter, approximately 100µL of settled sample extract is applied to sample port, where it will begin to react with gold particles conjugated to the polyclonal antibodies. The mixture will then migrate across the membrane thus enabling visualization of the test line (T) and procedural control (C). The test outcome is interpreted by visualizing the appearance of these lines at 10 or 15 minutes based on desired LOD.

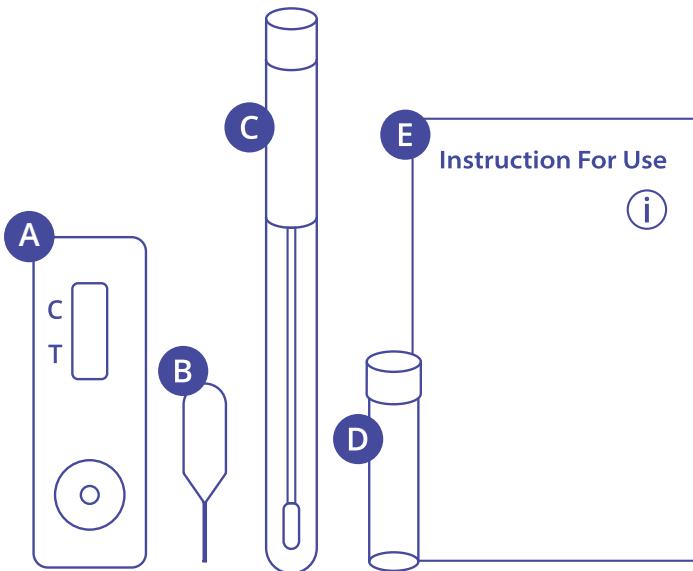


5. Kit Storage and Safe Handling

- ★ Store in original packaging at 2-8°C (36-46°F). 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.

6. Kit Components

- A OnSite® LFD devices
- B Single-use droppers
- C Breakaway swabs
- D Pre-measured OnSite® LFD Extraction Buffer
- E Instructions For Use



6.1. Optional additional materials

- Laboratory timer
- Calibrated laboratory pipette for measuring liquids
- Fine-tipped marking-pen
- Lateral flow device reader
- Non-powdered disposable gloves

7. Rinsewater Sampling and Analysis

7.1. Sample preparation and analysis



IMPORTANT: Prior to starting, ensure ALL test kit components have been brought to room temperature!

7.1.1. Prior to beginning, 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 taken from rinsewater. Increasing the number of samplings performed will ensure results are a more accurate representation of current conditions. As a best practice, hold a reserve sample of 20mL in case repeat testing is warranted.

7.1.3. Use an enclosed single-use dropper to transfer 5 drops of rinsewater to a vial of extraction buffer.

A precise sample size is critical for accurate results. For increased accuracy, use a calibrated pipette to transfer 100µl of rinsewater to a vial of extraction buffer.

7.1.4. Replace the lid tightly. Shake vial vigorously for two minutes.

7.1.5. Open a new lateral flow device and set it on a flat, level surface.

7.1.6. Open the extraction tube and withdraw sample extract using either a provided single-use dropper or a pipette calibrated to 100 μ L.

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.

7.1.7. Apply either 5 drops from the single-use dropper or 100 μ L from the calibrated pipette of sample extract to the sample port of the lateral flow device.

7.1.8. Observe test results promptly at 10 minutes for an LOD of 5 mg/kg (ppm) or at 15 minutes for an LOD of 3 mg/kg(ppm).

NOTE: To ensure accuracy and to avoid misinterpretation of the drying artifacts, analyze results promptly at either 10 or 15 minutes.

7.2. Interpreting test results

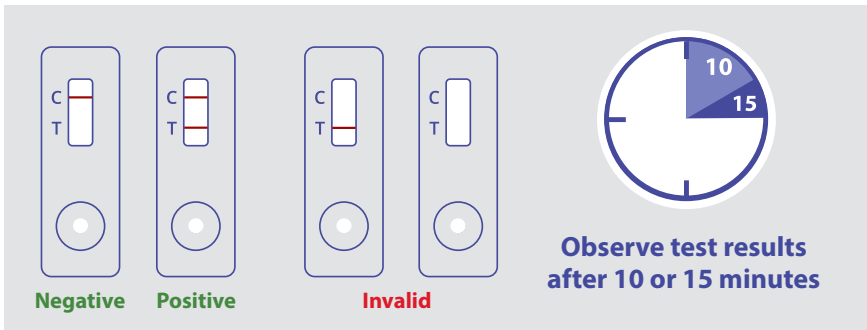


Figure 1. Test Outcomes

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 1** 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 tree nut protein present in the sample; any visible line is considered to be a positive result. An example of a positive result is displayed in **Figure 1** 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 1** labeled “**Invalid**”. In the event of an invalid test, the procedure should be repeated using a new test device.

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8. Environmental Sampling and Analysis

8.1. Sample preparation and analysis



IMPORTANT: Prior to starting, ensure ALL test kit components have been brought to room temperature!

8.1.1. Prior to beginning, thoroughly clean hands to reduce the risk of allergen contamination. Disposable gloves may be used. Open a new sampling swab and moisten the swab tip with tap, bottled or RO water. If water is unavailable, the provided extraction buffer may be used.

8.1.2. Collect a surface sample by swabbing a 4x4 inch (or 10x10cm) area using a rolling crosshatch technique shown in **Figure 2**. Ensure that all sides of the swab bulb come into contact with the surface.

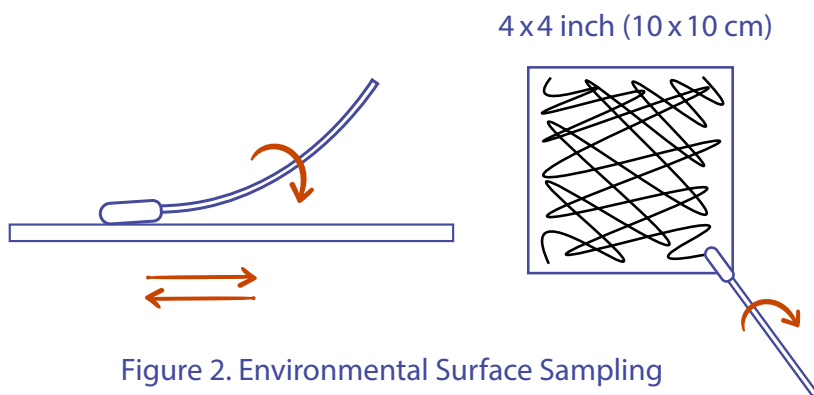


Figure 2. Environmental Surface Sampling

8.1.3. Open a vial of extraction buffer and place the swab tip into the extraction buffer. Carefully snap the swab at the breakaway point by pressing the swab against the wall of the vial.

8.1.4. Replace the lid tightly. Shake vial vigorously for two minutes.

8.1.5. Open a new lateral flow device and set it on a flat, level surface.

- 8.1.6.** Open the extraction tube and withdraw sample extract using either a provided single-use dropper or a pipette calibrated to 100µL.
- 8.1.7.** Apply either 5 drops from the single-use dropper or 100µL from the calibrated pipette of sample extract to the sample port of the lateral flow device.
- 8.1.8.** Observe test results promptly at 10 minutes for an LOD of 5 mg/kg (ppm) or at 15 minutes for an LOD of 3 mg/kg(ppm).

8.2. Interpreting test results

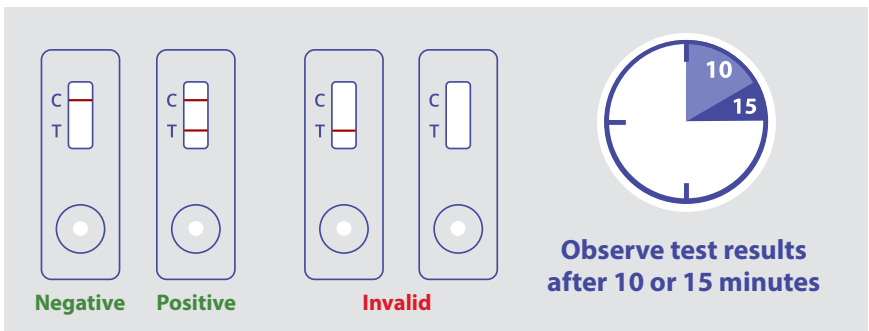


Figure 3. Test Outcomes

8.2.1. 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**”.

8.2.2. 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 tree nut protein present in the sample; any visible line is considered to be a positive result. 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® rapid tests are designed to detect native and processed allergen residues. However, detection of highly processed (autoclaved or extensively hydrolyzed) or high-fat samples may be less efficient. The test may underestimate target allergen in matrices that have extensive polyphenolic compound content (e.g. seasoning and spices) or those having undergone extensive chemical or thermal processing.

Furthermore, OnSite® Allergen Multiplex Tree Nut tests are not intended for high-analyte concentrations. Signal will be faint but remain evident at 1,000 mg/kg (ppm) protein in rinsewater. For contamination levels above 1,000 mg/kg (ppm) in rinse water, increased dilution or ELISA analysis is suggested.

OnSite® Allergen Multiplex Tree Nut tests show a weak cross-reactivity with kidney bean, rolled oats, poppy seeds, and rice flour and a significant cross-reactivity with coconut. Coconut protein residues in rinsewater above 50mg/kg (ppm) may prompt positive test results.

OnSite® Allergen Multiplex Tree Nut tests have been validated against many common food matrices with no cross-reactivity found. Validated matrices include buckwheat, chickpea, cocoa powder, cornmeal, milk, peanut, protein shake, quinoa, soy flour and wheat flour. Full validation data is available on request.

OnSite® Allergen Multiplex Tree Nut tests have been additionally validated against sanitizing agents containing peroxide, alcohol and ammonium salts with no interference noted at approximate working-strength levels.

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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 or 15 minutes.
- 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.
- All tolerances for this assay are temperature $\pm 5^{\circ}\text{C}$, volumes and weights $\pm 1\%$.
- Avoid using powdered gloves as this may introduce unwanted allergens.
- 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 target allergen 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: info@onsitefoodsafety.com.

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

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