

AlerTox® Sticks

Mustard Seeds

Immunochromatographic rapid test for qualitative detection of mustard seeds antigen in food and surfaces.

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1. Intended use

AlerTox Sticks Mustard Seeds is an immunochromatographic rapid test for qualitative detection of mustard antigen in food and surfaces.

2. Introduction

Mustard is a condiment made from the seeds of the mustard plants (white or yellow mustard, *Sinapis alba*; brown or Indian mustard, *Brassica juncea*; or black mustard, *Brassica nigra*). Oriental mustard, also called Chinese mustard or Jie Cai/Gai Choy (or *Sisymbrium orientale*) is another member of the mustard family, as well as the wasabi plant (*Eutrema japonicum*).

Mustard allergy can display a variety of symptoms from mild oral allergy or hives to severe life-threatening systemic reactions, i.e. anaphylactic shock or bronchial asthma. Allergy to mustard is one of the major food allergies in some geographical areas, though there is no estimation of overall global prevalence percentage. Mustard and products thereof are listed in Annex II of Regulation (EU) No. 1169/2011 on labelling of foods.

3. Test sensitivity and specificity

AlerTox Sticks Mustard Seeds uses a unique combination of monoclonal antibodies against a major mustard seed antigen, the legumin-like 11S seed storage protein (cruciferin, CRU4), known as allergen Sin a 2. AlerTox Sticks Mustard Seeds detects the antigens of all mustard varieties, including wasabi. **AlerTox Sticks Mustard Seeds is highly cross-reactive with seeds of species of the cruciferous family (broccoli, Brussels sprouts, cabbage, cauliflower, horseradish, radish, rutabaga, turnip).** The test is non-reactive to the edible parts (leaves and roots) of other species of the cruciferous family as well as rapeseed/canola oil.

The LOD (limit of detection) of AlerTox Sticks Mustard is 2 ppm of raw, unprocessed mustard seeds protein.

PLEASE NOTE: The sensitivity of the test decreases with heating of the food (cooking) at temperatures exceeding 150 °C (302 °F) and in fat-rich environments (e.g. in presence of oil or cream), in matrices with a low pH (e.g. vinegar or citric acid), or when performing the test at temperatures below 18 °C (64.4 °F).

The range of detection (ROD) is 5-1,000 ppm. Above this range, the test can present a hook effect. The hook effect can appear as a negative result or a test line with reduced intensity. If a false negative due to hook effect is suspected, repeat the test on a diluted sample.

4. Kit contents

- 10 immunochromatographic sticks individually packed in foil pouches
- 10 sample collection tubes (tube with yellow cap)
- 10 sample extraction buffer tubes, 10 mL (tube with blue cap)
- 10 spoons
- 10 pipettes (3 mL- only for testing liquid samples)
- 10 small pipettes
- 10 swabs (only for testing surfaces)
- Instructions for use

5. Other materials not supplied

- Grinder, mortar or any other manual or automatic homogenization system to crush the sample
- Scissors
- Optional: digital scale sensitive to 0.1 g

6. Precautions

- The test sticks must be stored at a temperature between 10 °C and 30 °C (50 °F and 86 °F).
- Use the test within 10 minutes after opening the foil pouch.
- Do not touch the white end of the stick.
- Do not use the test stick when its pouch is torn, or the stick is broken or damaged.
- All the components of the test kit are disposable; do not reuse them.
- Do not use the test sticks beyond the expiry date.

7. Sample handling

The samples must be brought to a temperature between 18 °C and 35 °C (64.4 °F and 95 °F) before use. The test is designed to detect the target antigen in:




- Solid food.
- Liquid samples: beverages, washwater from cutting equipment and surfaces used in food processing and storage.
- Surfaces.

8. Test procedure for solid foods

8.1. Before opening the foil pouch containing the test stick, please leave it at room temperature while you process the samples.

8.2. Mash or crush the sample to obtain the finest crumbs possible. Use a mortar or a grinder if possible.

8.3. Use a scale to weigh 1 g of the sample, or follow the chart below to add an equivalent amount of sample to a yellow-capped tube, using one of the single-use spoons provided.

| Food type | Examples | Spoonfuls |
|---------------------------|--|---|
| Flours, fine powders | Corn flour, rice flour, milk powder, spices, etc. |  |
| Fine crumbs | Bread, cookies, cakes, snacks, etc. |  |
| Meat, fish and cured meat | Meat, fish, sausage, black pudding, pate, canned meat and fish, etc. |  |

8.4. Pour the entire content of a blue-capped tube (10 mL) into the yellow-capped tube.

Keep the blue cap, as it will be used later on.

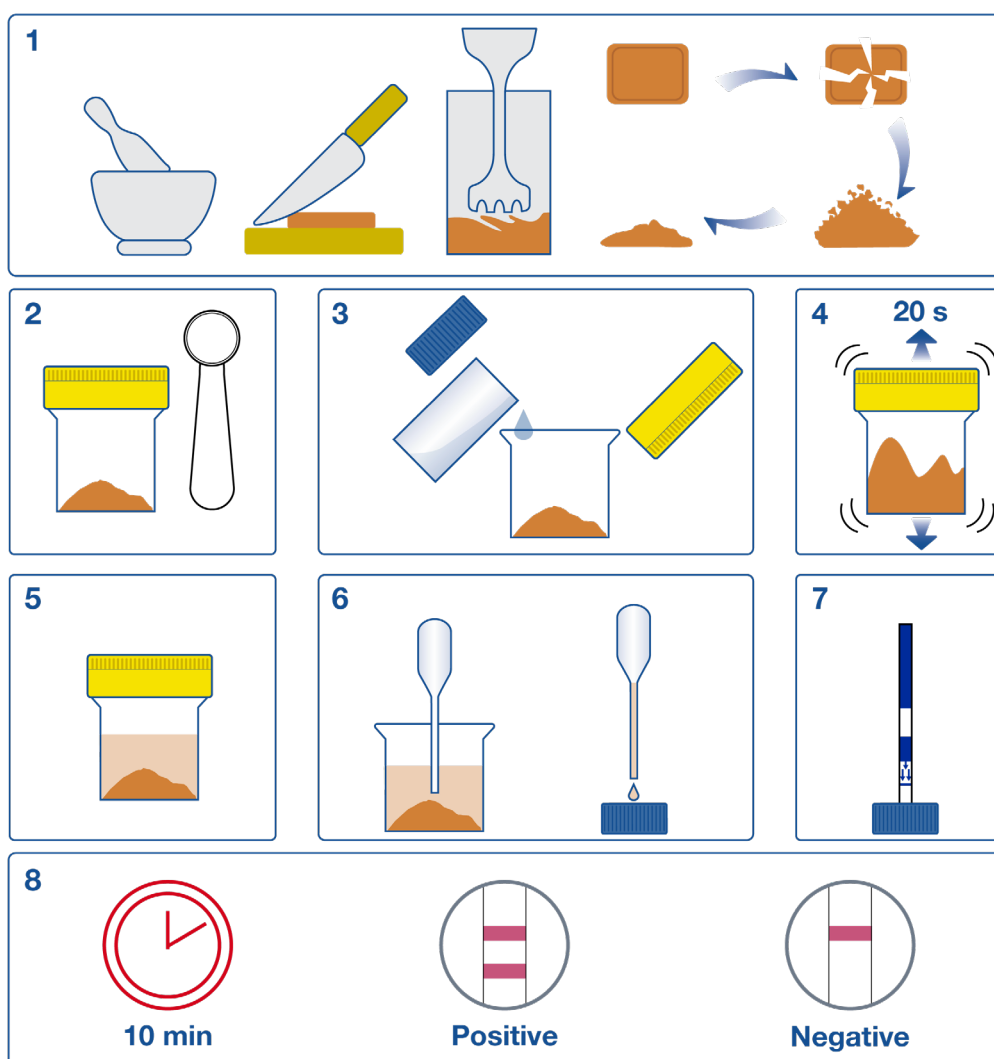
8.5. Close the yellow-capped tube and shake it vigorously for at least 20 seconds. Let it rest for 2 minutes so the solids settle.

8.6. With a small pipette, transfer supernatant to the blue cap until it is full.

8.7. Open the envelope and pull out the stick carefully, by holding its BLUE end. Do **NOT** touch the white end of the stick.

8.8. Place the white end of the stick in the blue cap and wait 10 minutes to read the result. Do not leave the stick longer than indicated, as the results may vary. Do not touch the stick while waiting.

Test procedure for solid foods



9. Test procedure for liquid samples

Liquid samples – beverages, washwaters from kitchen dishes, technological surfaces or cutting machines – may be tested directly. Turbid samples should be filtered (paper or textile filter) or allowed to settle.

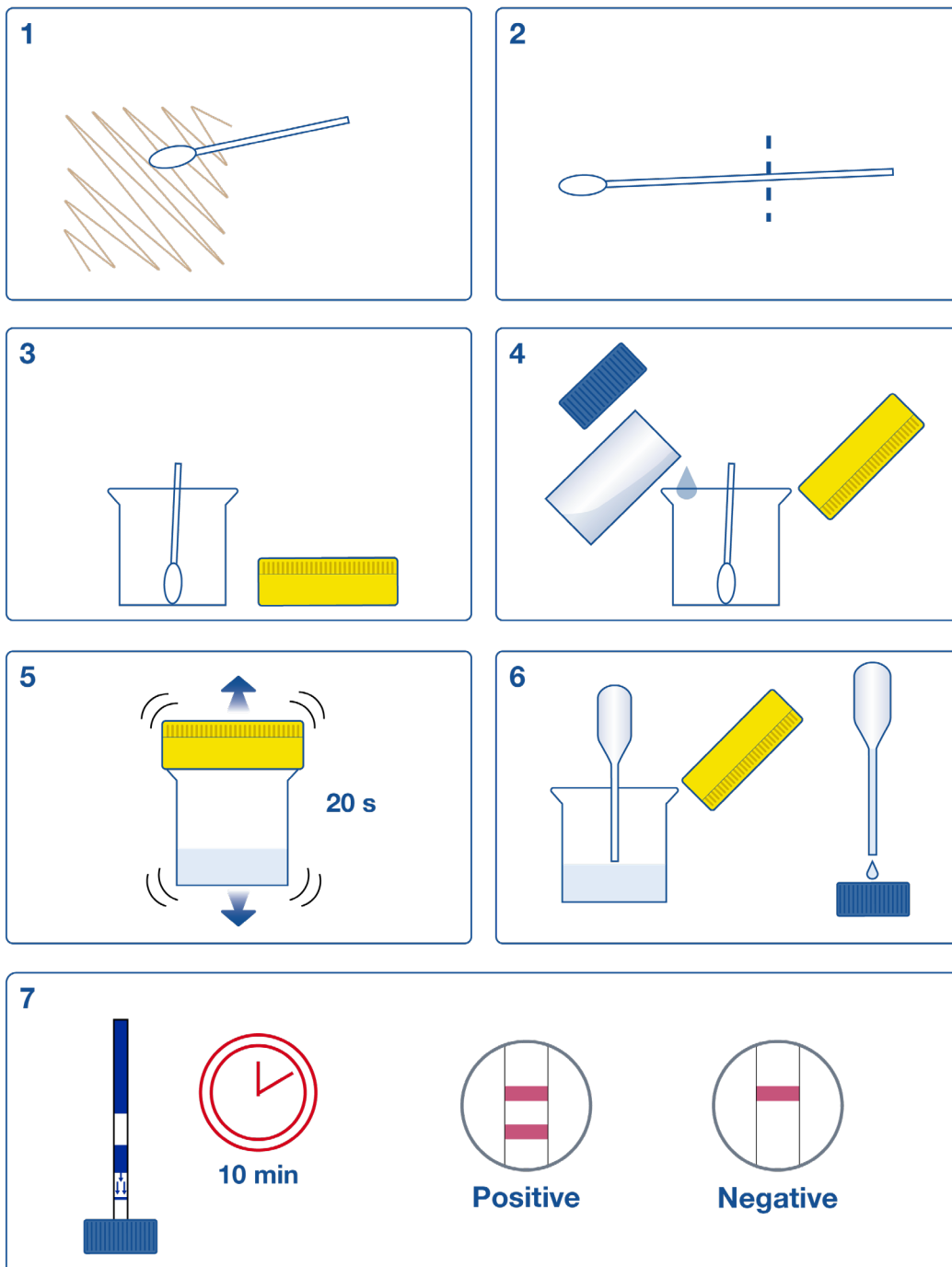
- 9.1.** Before opening the foil pouch containing the test stick, please leave it at room temperature while you process the samples
- 9.2.** Using a provided 3 mL pipette, add 3 mL of your liquid sample to a yellow-capped tube. If the sample is thick (e.g., yogurt, sauce, etc.), follow the chart below to add an equivalent amount of sample to the yellow-capped tube, using one of the single-use spoons provided.
- 9.3.** Add an equal volume of sample extraction buffer (3 mL) using the same pipette, screw the yellow cap and mix by gently shaking the tube for at least 20 seconds. If the liquid is cloudy, let it settle.
Keep the blue cap, as it will be used later on.
- 9.4.** With a small pipette, transfer supernatant to the blue cap until it is full.
- 9.5.** Open the envelope and pull out the stick carefully by holding its BLUE end. Do **NOT** touch the white end of the stick.
- 9.6.** Place the white end of the stick in the blue cap and wait 10 minutes to read the result. Do not leave the stick longer than indicated, as the results may vary. Do not touch the stick while waiting.

| Food type | Examples | Spoonfuls |
|-------------------|--|-----------|
| Liquid and sauces | Milk, juice, condensed milk, yogurt, soup, gravy, sauce, cream, etc. | |

10. Test procedure for surface analysis

- 10.1.** Firmly rub the swab on the surface that is going to be analyzed (at least 16 cm²/2.46 in², or a line of 40 cm/15.6 in. The area selected for analysis must be representative of the total area of interest.
- 10.2.** Introduce the swab into the sample collection tube and, using scissors, trim the swab so that it will fit in the yellow-capped tube with the cap closed.
- 10.3.** Pour the entire content of a blue-capped tube (10 mL) into the yellow-capped tube.
Keep the blue cap, as it will be used later on.
- 10.4.** Vigorously shake the tube for at least 20 seconds.
- 10.5.** With a small pipette, transfer supernatant to the blue cap until it is full.
- 10.6.** Open the envelope and pull out the stick carefully by holding its BLUE end. Do **NOT** touch the white end of the stick.
- 10.7.** Place the white end of the stick in the blue cap and wait 10 minutes to read the result. Do not leave the stick longer than indicated, as the results may vary. Do not touch the stick while waiting.

Test procedure for surface analysis



11. Interpretation of results

The result of the test is POSITIVE if TWO colored lines appear: One in the control zone (C) and one in the test zone (T).



The result of the test is NEGATIVE if only ONE colored line is clearly visible, in the control zone (C).



If NO colored line appears in the control zone (C), the test is INVALID.



In the case of an invalid test, repeat the test with another stick, check the correct specimen handling and test procedure, expiry date and storage conditions. Contact your distributor for further details.

IMPORTANT NOTE!

- **AlerTox Sticks is a qualitative test intended for the screening of samples for internal quality control. Under no circumstances can it replace the quantification test of the laboratory analysis.**

12. Validation

AlerTox Sticks Mustard Seeds has been validated for the following matrices:

- Mustard seeds (yellow mustard seed, brown mustard seed, black mustard seed and oriental mustard seed)
- Indian Spice mix
- Spice Mix for sausages (salt, vinegar, ground pepper, paprika, cayenne peppers, ground chili, cumin, brown mustard seeds)
- Flour (Mustard flour, yellow mustard flour, ground brown mustard, cracked yellow mustard seed, cracked brown mustard seed, mixed mustard bran, yellow mustard bran, brown mustard bran)
- The test has resulted positive but with a decrease in sensitivity in the following matrixes: pure oriental mustard and ground yellow mustard