Disp&FLOW® – Mustard

Rapid test for the detection of specific protein

N° cat.: BIO.038.25

Number of test: 25

Rapid immuno-chromatographic test for the qualitative determination of mustard-specific protein in in foods, kitchens and food production facilities.

The Disp&FLOW - Mustard test has been designed to detect the target antigen in two main types of specimens:

1. Solid food samples / solid food products / kitchen utensils / preparation surface.
2. Liquid samples of the following types: drinks; rinsing water from food preparation tools and kitchen equipment; washing water from surfaces used for cutting, processing and storing food products.

How it works

Mustard is a condiment made from the seeds of the plant of the same name (white or yellow mustard, *Sinapis alba*; brown or Indian mustard, *Brassica juncea*; or black mustard, *Brassica nigra*), the two most important being white and black. Oriental mustard (Chinese mustard or *Sisymbrium orientale*) is another member of the mustard family, as is Wasabi (*Eutrema japonicum*).

Allergies to mustard manifest themselves through a variety of symptoms, from limited eczema or urticaria-type allergy to more or less severe systemic reactions such as rhinitis and bronchial asthma, or potentially fatal ones such as anaphylactic shock. Mustard allergy is common (ranked in the top 10) among food allergies in certain geographical areas, but there are no reliable estimates of the overall prevalence of this allergy and its evolution. Mustard and its derivatives are included in the European Directive 2007/68/EC on the labelling of allergenic foods, on the list of ingredients to be taken into account (Annex IIIa).

The Disp&FLOW - Mustard test detects one of the major allergens in mustard seeds: cruciferin CRU4 (11S-globulin, allergen Sin a 2 - storage protein). This cooking-resistant protein is a member of the legumin family, and shows a certain homology with similar allergens found in other plants (soy, peanut, Brazil nut and cashew nut).

The Disp&FLOW - Mustard test is based on the principle of rapid immuno-chromatography on a strip (lateral flow migration). The target antigen present in the sample is absorbed by the strip and then recognized by specific antibodies conjugated to colored microparticles that are free to move. Once formed, this complex migrates along the strip to a highly focused area where it meets another specific antibody attached to the support. The accumulation of microparticles quickly forms a colored line indicating a positive result. The presence of a second control line guarantees that the test is working properly.

Test specificity and sensitivity

The Disp&FLOW - Mustard test uses a pair of carefully selected, high-performance monoclonal antibodies to detect the mustard target antigen using the rapid immuno-chromatographic technique. This test detects the antigens of all mustard varieties but does not detect the presence of Wasabi. The test is non-reactive with the other plants tested: vegetables, nuts, spices and cereals. Similarly, no cross-reactivity was observed with the edible parts (leaves and roots) of other species in the Brassicaceae family - cabbage, broccoli, Brussels sprouts, cauliflower, horseradish, radish, rutabaga, turnip - or with rapeseed oil (or canola oil).

IMPORTANT TO NOTE: the Disp&FLOW - Mustard test is highly reactive (cross-reactive) with the seeds of the plants mentioned above, which are not generally used in the food industry.

The sensitivity of the Disp&FLOW - Mustard test is approximately 5 ppm, measured during a typical extraction, on the dry weight of crushed, unprocessed mustard seeds. The test detection range is between 5 and 100,000 ppm. The sensitivity of the Disp&FLOW - Mustard test decreases as the food is modified by heating (cooking) at temperatures exceeding 120°C.

If the test result is a faintly colored or unclear line, BIOTEM recommends retesting the sample with a different method, such as a quantitative ELISA.

Kit content

The Disp&FLOW - Mustard test contains the following elements:

- 1 tube with desiccant cap containing 25 test strips.
- 25 sample collection swabs.
- 25 polypropylene test tubes containing 3 mL extraction buffer for sample preparation and test migration.
- 25 transfer pipettes.
- Instructions for use.

Storage and stability

- The kit should be stored between +2 and +30°C in a dry atmosphere away from direct sunlight.
- **The strips should not be frozen and should be kept in their hermetically tube.**
- The kit must be used before the expiry date indicated on the packaging.

Equipment required but not supplied

- Sampling spatula, preferably single-use.
- Pair of gloves

Precautions

- The kit components are for *in vitro* use only.
- The kit can be used until its expiry date if it has been stored under the recommended conditions.
- Do not use the test after its expiry date.
- The tube containing the test strips should be stored between +2 and +30°C.
- All handling associated with the use of this test must be carried out in strict compliance with the conditions for non-contamination of samples; in particular, gloves must be worn during handling.
- The strips should be handled by their colored upper part. Do not directly touch the central part of the strip or its absorbent end.
- The strips must be stored in their hermetically tube (the strips are very sensitive to humidity) - do not use a strip more than 10 minutes after opening the tube.
- Only use the tube containing the extraction buffer supplied in the kit. Never use components from different kits.
- Close the tube immediately after removing the required number of strips needed.
- Do not use the test if the strip tube has been found open.
- Take care not to immerse the strip deeper than the line under the arrows.

- The Disp&FLOW - Mustard test contains only single-use components; do not use again.

Waste disposal

- Dispose of all used consumables in accordance with biomedical waste regulations.
- All users are responsible for managing the waste they produce and for ensuring that it is disposed of in accordance with applicable regulations.

Preparation of samples

Prior to testing, samples should be brought to a temperature between +18°C and +35°C; analysis of colder samples reduces the sensitivity of the test; analysis of warmer samples is not possible due to the risk of degradation of the antibodies present in the strip. Make sure that the material to be tested is a mixture of all the ingredients that make up the final solid food product.

Liquid samples can be tested directly.
The test detection limit for liquid samples depends on their viscosity and turbidity (presence of particles).
If the sample is viscous and cannot reach the test zone, it must be diluted in the dilution/extraction buffer. In this case, the sensitivity of the test must be adjusted by the dilution factor.
Cloudy specimens should be filtered through a textile or paper filter.

1. Using the transfer pipette supplied, insert the liquid sample (1-2mL) into the test tube containing 3 mL of dilution/extraction buffer.
2. Hermetically seal the tube with the stopper.
3. Vigorously shake the tube manually or using a vortex at maximum speed for 20-30 seconds.
4. Place the tube vertically on a stand and allow the contents to settle or centrifuge at low speed in a centrifuge, the supernatant is then ready for testing.

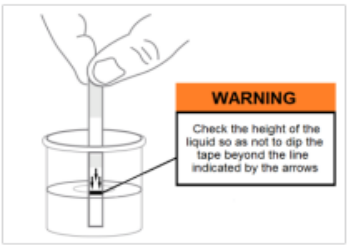
Powdered products must be tested using the following procedure:

1. Using clean, sharp tools (preferably disposable), cut a small piece (0.1 to 0.5 g) of the material to be tested and introduce it into the tube containing the dilution/extraction buffer.
2. For utensils or other surface tests, use the swab provided and pass it over the surface of the object to be analyzed in a criss-cross motion, first in one direction, then in the other, then diagonally. Then insert the swab into the test tube and shake for 10 to 15 seconds in the extraction buffer.
3. Hermetically seal the tube with the stopper.
4. Vigorously shake the tube manually or using a vortex at maximum speed for 25-30 seconds.
5. Place the tube vertically on a stand and allow the contents to settle or centrifuge at low speed in a centrifuge, the supernatant is then ready for testing.

Individual specimens can be stored at +2°C to +8°C for up to 24 hours. For longer storage, we recommend freezing the specimen.

Test procedure

1. Bring the samples to a temperature between +18 and +35°C.
2. Remove the tube containing the test strips (without opening it), the extraction buffer and leave it at room temperature for 5 to 10 minutes.
3. Open the tube, grasp a strip by the top colored part and dip the other end vertically into the supernatant in the test tube and immediately close the tube with its desiccant cap. Caution: make sure that the strip is not immersed too deeply - see diagram opposite ➔



4. Soak the strip for **20 to 30 seconds** and then place it on a CLEAN, horizontal surface; do not touch or move the strip for **10 minutes** while the sample migrates.
5. Read the result and interpret it in accordance with the diagrams and instructions below.

Interpretation of results

The test is positive if 2 red lines appear clearly in the central area of the strip (test line and control line, see below). Ignore the order in which the 2 lines appear and any nuances in color intensity.

The test is negative if a single red line appears (see below): this is the control line which guarantees that the test is working correctly.

If only the test line appears (see below), the test cannot be interpreted, and no result is validated.

If no line appears (see below), the test cannot be interpreted, and no result is validated.

In the latter two cases, before starting again with another Disp&FLOW - Mustard test, it is important to ensure that all the test preparation, storage and implementation instructions have been followed, as well as the expiry date.

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