

Mustard Free Test

LATERAL FLOW TEST KIT

for the detection of mustard residues in food, cip solutions and working surfaces

ProGnosis Biotech S.A. is ISO 9001:2015 certified by TÜV Hellas (TÜV NORD).

Use only the current version of Product Data Sheet enclosed with the kit.

Mustard Free test, E2110/E2130, is a lateral flow test that detects mustard residues in food products, cip solutions and working surfaces. The lateral flow kit contains all reagents required for the immunoassay method.

Matrices:

Cream cheese, Condiments, Dressings, Instant Soup, Ketchup, Meats, Mixed herbs, Sausage, Spices

- Sample preparation: extraction/swab sampling
- Test time (incubation time after samples and reagents preparation): 5 min
- Calibration Range: 3-128ppm
- Shelf life: 12 months
- Storage: 4-30^{o}C

Method characteristics

- The LOD of the method is 2.0 ppm (2.0mg/kg) mustard flour or 0.52 ppm mustard proteins in food samples and CIP solutions.
- The LOQ of the method is 3.0 ppm (3.0mg/kg) mustard flour or 0.78 ppm mustard proteins in food samples and CIP solutions.
- Limit of detection (LOD): 0.1μg/100cm^2 on working surfaces. LOD was calculated based on our reference materials.
- The target indicator protein of the antibody is a major mustard allergen and was selected based on its strong resistance to food processing and high abundance in the mustard seed.

1. Description

Mustard Free test is a Lateral Flow test for the detection of mustard protein residues in food products, specially for those labeled as mustard-free, CIP solutions and working surfaces.

2. General Information

Mustard (*Sinapis alba, Brassica juncea, Brassica nigra*) belongs to the Brassicaceae. It contains a high fraction of of proteins (30-35%) and many of these are known to be allergenic, such as Sin a 1 and Bra j 1, among others. Many of these proteins are thermally-stable during various production processes. Consumption of mustard might be harmful for people who are allergic to it. The allergen can be present as an ingredient or as a contamination in raw and cooked products. Consumption of mustard-containing food from allergic people might cause a broad range of symptoms, such as hives, mild oral allergy or/and anaphylactic shock. Because of this, consumption of mustard or mustard-containing food should strictly be avoided from allergic persons. According to the regulation (EU) No. 1169/2011 Annex II, mustard is included in the list of allergens established by the European Food Safety Authority, and its presence must be indicated on the label. Similar regulations exist e.g. in the USA, Canada, Australia and New Zealand.

3. Principle of the method

The presence of mustard in a sample is determined by the immunological detection of mustard proteins. Antibodies specific to mustard proteins are coated on the test line region (Test line) of the nitrocellulose membrane. During testing, antigens in the specimen react with the antibodies that are coated onto gold nanoparticles. The mixture migrates up the membrane to react with the antibodies immobilized on the membrane and generate a colored line in the test region T. The presence of the colored Test line indicates a positive result. In case of samples with a very high allergen concentration, the Test line fades or may not appear, giving us reduced or false negative result (hook effect phenomenon). For this purpose, a second line has been created (Hook line), whose intensity decreases as the amount of antigen increases and at very high concentrations it disappears either together with the Test line or before it. To serve as a procedural control, a colored line will always appear in the control region (Control line) if the test has been performed properly.

4. Reagents Provided

Reagents (Store at 4-30ºC)	E2110	E2130
Reaction device	10pcs	30pcs
Prefilled sample tube with white cap	10pcs	30pcs
Disposable pipettes	10pcs	30pcs
Prefilled Extraction tube with dropper tip	10pcs	30pcs
Sterile swab	10pcs	30pcs
Instruction manual	1	1

5. Materials required but not provided

- A grinder sufficient to render sample to particle size of fine instant coffee
- Balance with 0 - 50 g measuring capability
- Microcentrifuge and centrifugal vials
- Vortex mixer and/or Shaker
- S-Flow Reader or 3PRMini along with matching software (for quantitative detection)

6. Storage Instructions

Store kit reagents between 4 and 30°C (39.2 - 86°F). Do not freeze any components provided. Expiry of the kit and reagents is stated on the labels respectively and no quality guarantee is accepted after the expiration date. The expiry of the kit components can only be guaranteed if the components are stored properly as well as if the reagent is not contaminated by the first handling, in case of repeated use of one component. Do not interchange individual reagents between kits of different lot numbers.

7. Safety and Precautions for use

- Use gloves and disinfect the workbench before starting
- All reagents should be warmed in room temperature before use and covered when not in use. Use a clean disposable pipette for each sample, in order to avoid cross-contamination.
- Clean surfaces, glass vials, mincers and other equipment before and after each sample preparation.
- Do not mix and interchange different samples.
- Do not interchange individual reagents between kits of different lot numbers
- Do not re-use any of the kit components as they are single-use only
- Do not eat or drink in the area where the samples and the kit are stored and handled.

8. Samples Preparation

8.1 Solid Samples

1. The sample must be collected according to established sampling techniques. Grind a representative sample (at least 5 g) to the particle size of fine instant coffee (50% passes through a 20 mesh screen).
2. Weigh out a 0.5 g ground portion of the sample, add it into the prefilled sample tube and vortex it for 1min. **The ratio of sample to extraction solvent is 1:10 (w/v).**
3. Two (2) ml of the extract should be centrifuged at high speed for 2 min in reaction caps by using a microcentrifuge. Alternatively, let the sample settle down.
4. Using a disposable pipette, transfer 3 drops from the supernatant to the reaction device and allow test to develop for 5 minutes.

NOTE 1: The extracted sample should have a pH value of 6.2 - 7.5. If the pH is less than 6.2 or more than 7.5, the pH should be neutralized using NaOH or HCl.

NOTE 2: In case of cloudy, thick samples, that do not allow the mixture to develop, a dilution 1:1 with the extraction buffer is required before transferring 3 drops to the reaction device. In this case, multiply the final mustard proteins ppm result x 2.

8.2 Liquid Samples and CIP Solutions

1. Use 0.5 mL of the sample, add it into the prefilled sample tube and vortex it for 1min.
2. Using a disposable pipette, transfer 3 drops from the supernatant to the reaction device and allow test to develop for 5 minutes.

NOTE: In case your sample is a viscous liquid, you can proceed with the procedure as for solid samples.

8.3 Surfaces and swab sampling

1. Mark out a swabbing area of approximately 10 x 10 cm.
2. Moisten a swab by dipping into the extraction tube.
3. Gather the sample with the swab by using a crosshatch technique **(Figure 1.)**. Move the swab horizontally, vertically, diagonally while rotating the tip. Repeat this starting from a different angle each time.
4. After the sample collection, place the swab in the extraction tube, rotate the swab forcefully against the side of the tube for 1min. Best results are obtained when the sample is vigorously extracted in the solution. Remove the swab, squeezing the sides of the tube to extract as much liquid as possible. Shake vigorously for 1min on a vortex.
5. Close the extraction tube with the dropper tip. Add 3 drops in the circular window of the reaction device.

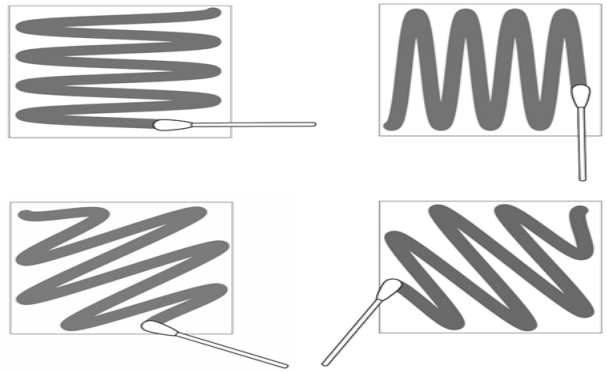


Figure 1.

9. Interpretation of results

9.1 Qualitative assessment

Note: For internal procedure purposes three colored lines are present on the result window of the Mustard Free Test. The colored lines have no effect on the product's performance since they are washed away during the experiment.*

After 5 minutes, the test device can be visually read and interpreted according to the following figure. Observation after 10 minutes lead to inaccurate conclusions.

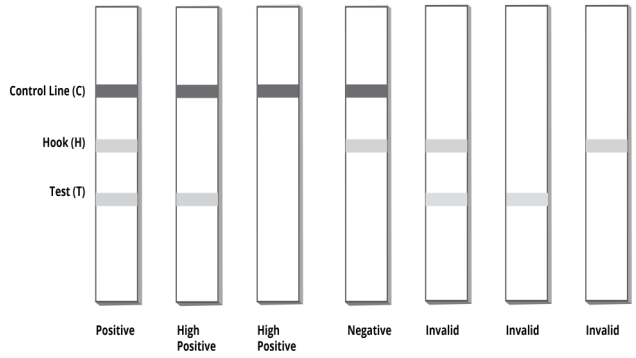
Negative Result: Two visible colored bands appear at both Hook line (H) and Control (C) line. It indicates that the concentration of the mustard is zero or below the detection limit of the test.

Positive Result: Three visible colored bands appear at Hook line (H), Control line (C) and Test line (T). Any intensity of Test line indicates the presence of mustard into the sample.

High Positive Result: No colored band is visible at Hook line (H) and the band at Test line (T) may be **faint** or absent. It indicates that the sample contains mustard to very high concentrations.*

Invalid Results: No colored band appears at Control line no matter whether it appears at Test line, a Hook line or not.

*In this case you can confirm the high content of your sample by diluting it . Add one drop with the disposable pipette in a new tube and shake vigorously. You are expected to get an image as **Positive Result** or **High Positive Result** with the presence of Test line. To quantify your result multiply by the dilution factor x 200.



Visual result interpretation index

9.2 Quantitative procedure

After 5 minutes, place the reaction device inside the plastic holder in order to be scanned. Use the appropriate software to quantify results as soon as possible and no later than 1 minute after the end of analysis. The software will use a Lot specific curve to calculate the results. Refer to the Reader's manual for a detailed description of the quantification procedure.

10. Performance Evaluation

10.1 Reference Materials

Several reference materials are being used for the evaluation of each product of ProGnosis Biotech S.A. in the context of Quality Control performed by the Quality Control Department. Please request a validation report, including the results, at info@prognosis-biotech.com.

11. Assay Claims

- Samples showing negative results may contain Mustard below the limit of detection of the assay. This Lateral Flow kit does not claim that food is safe for consumption based upon a determination of mustard content. Matrix effects may also affect the result of the method.
- The recovery/cross reactivity of the method might be affected when analyzing processed food (e.g. heat treatment, dehydration, etc.), because proteins may be altered or fragmented.
- Food samples that have been heat treated may contain denatured proteins which may not be captured by the antibody. Recovery of these matrices might be reduced.
- The protein content and the protein composition may differ among various species of the same matrix. Therefore, different varieties may produce different results.
- LOD and LOQ in CIP solutions refer to the final rinse water. The presence of cleaning agents and detergents may affect the result of the method.
- Test results are expressed as ppm mustard flour. Mustard contains around 26% protein. To express results as ppm mustard protein, multiply the result by 0.26 (e.g., 1 ppm mustard x 0.26 = 0.26 ppm mustard protein).

12. Method Summary

Total procedure time (after samples and reagents preparation): 5 min.

12.1 Food samples and CIP solutions

Add 0.5gr or 0.5mL of the sample into the Prefilled Sample Tube

⇩

Vortex them for 1min. Let the sample settle down.

⇩

Centrifuge the sample for 2 min, at high speed in a microcentrifuge.

⇩

Transfer 3 drops from the supernatant to the reaction device

⇩

Allow test to develop for 5 minutes. Read the results immediately.

⇩

Read the results visually or place the device in Reader to be scanned

12.2 Working surfaces

Mark out a swabbing area of approximately 10 x 10 cm

⇩

Moisten a swab by dipping into the extraction tube.

⇩

Gather the sample with the swab by using a crosshatch technique

⇩

Place the swab in the prefilled tube to extract the sample

⇩

Close the extraction tube with the dropper tip. Add 3 drops in the circular window of the reaction device.

⇩

Allow test to develop for 5 minutes. Read the results immediately

⇩

Read the results visually or place the device in Reader to be scanned

All immune assays supplied by ProGnosis Biotech S.A., are warranted to meet or exceed our published specification when used under normal conditions in your laboratory. If the product fails during the stated period, a replacement product will be issued.

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VERSION N5

CAT.NUMBER: E2110/E2130
STORAGE: 4-30°C

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