

VERSION 5
CAT.NUMBER: E3210/E3230

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Multi Tree Nuts Free Test

LATERAL FLOW TEST KIT

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

ProGnosis Biotech S.A. is ISO 9001:2015 certified.

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

Multi Tree Nuts Free test, E3210/E3230, is a qualitative lateral flow test that detects tree nuts (almond, Brazil nut, cashew, hazelnut, pecan, pistachio and walnut) residues in food products, cip solutions and working surfaces. The lateral flow kit contains all reagents required for the immunoassay method.

Matrices:

Beverages, Cake, Cereals, Chocolate, Cookies, and Crackers

- Sample preparation: extraction/swab sampling
- Test time (incubation time after samples and reagents preparation): 5 min
- Limit of detection (LOD): 0.3-12ppm :0.05-2.0μg/100cm^2 (for surfaces)
- Shelf life: 12 months
- Storage: 4-30^{o}C

Note: *The same extract can, also, be used in all the products of* ***Allergen Free*** *series. If you want to check your sample for more than one allergen at the same time, you can use the same extract to test as many of the products you have available.*

Method characteristics

Multi Tree Nuts Free test can detect allergenic proteins from seven of the most common types of tree nuts (almond, Brazil nut, cashew, hazelnut, pecan, pistachio and walnut), either individually or in combination, by using two detection (Test) zones.

- The table below shows the detection limits (LOD) for each of the seven tree nuts, in food samples, CIP solutions and working surfaces. LODs and LOQs were calculated based on our reference materials.

Tree nut	LOD (food, CIP)	LOD (surface)
Almond	0.3ppm	0.05µg/100cm^2
Brazil nut	10ppm	1.5µg/100cm^2
Cashew	3ppm	0.4µg/100cm^2
Hazelnut	4ppm	1.0µg/100cm^2
Pecan	10ppm	1.2µg/100cm^2
Pistachio	12ppm	2.0µg/100cm^2
Walnut	4ppm	1.0µg/100cm^2

- **Cross reactivity**

When cashew is present in the sample, with a concentration ≥ 100 ppm, a band can also be observed in Test line 2.

When Brazil nut is present in the sample, with a concentration ≥ 400 ppm, a faint band can also be observed in Test line 1.

When pistachio is present in the sample, with a concentration ≥ 1000 ppm, a faint band can also be observed in Test line 1.

***Cross-reactivity was tested up to a level of 10,000 ppm. In whole nuts, results may be different. For more information please request a validation* report.**

1. Description

Multi Tree Nuts Free test is a qualitative Lateral Flow test for the detection of tree nuts protein residues in food products, specially for those labeled as tree nut-free, CIP solutions and working surfaces.

2. General Information

The term "tree nuts" refers to a group of fruits with a hard shell that encases the edible kernel and grow on trees. Includes species like Almond, Hazelnut, Brazil nut, Cashew, Pecan, Pistachio and Walnut, some of which are closely related. Tree nuts are a good source of fiber, protein, vitamins, and other nutrients but, consumption from people who are allergic to them (one or more), might be harmful. Tree nut allergy is one of the nine most common food allergies, affecting roughly 0.5 to 1% of the U.S. population. The majority of proteins involved in tree nut allergy belong to protein families of 2S albumins, vicilins and legumins. Symptoms can vary from hives, nausea, difficulty of breathing to anaphylactic shock. The combination of their widespread use in the food industry and their allergenic effect has led companies to implement strict cleaning rules, such as Clean-in-Place (CIP) cleaning, applied to machinery and workplaces. According to the regulation (EU) No. 1169/2011 Annex II, tree nuts are included in the list of allergens established by the European Food Safety Authority, and their 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 a tree nut or more in a sample is determined by the immunological detection of tree nuts proteins. Antibodies specific to tree nuts proteins are coated on the test line region 1 (Test line 1) and Test line region 2 (Test line 2) of the nitrocellulose membrane. Test line 1 includes antibodies specific to brazil nut, pistachio and cashew (Group 1) while Test line 2 includes antibodies specific to almond, hazelnut, pecan and walnut (Group 2). 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. 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)	E3210	E3230
Reaction device	10pcs	30pcs
Prefilled sample tube with screw cap (5ml)	10pcs	30pcs
Disposable pipettes	10pcs	30pcs
Prefilled Extraction tube with dropper tip (0.5ml)	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

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. Alternatively, shake vigorously by hand. **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 7.2 - 8.5. If the pH is less than 7.2 or more than 8.5, 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.

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. Follow the rest of the procedure as in step **8.1.3.**

NOTE: You can skip using the microcentrifuge, unless your sample is a viscous liquid.

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 it on a vortex for just a second or you can just tip it with your fingers 3-4 times for better homogenization.
5. Close the extraction tube with the dropper tip. Add 3 drops in the circular window of the reaction device.

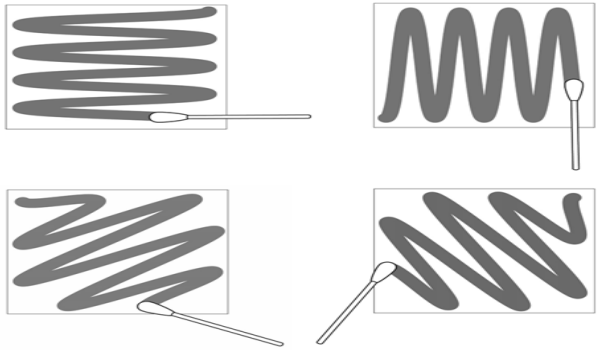


Figure 1.

9. Interpretation of results

9.1 Qualitative assessment

Note: For internal procedure purposes three colored lines are present on the result window of the Multi Tree Nuts 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 7 minutes lead to inaccurate conclusions.

Negative Result: One visible colored band appears at Control (C) line. It indicates that the concentration of tree nuts is zero or below the detection limit of the test.

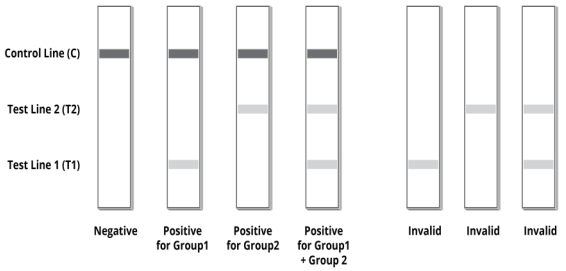
Positive Result for Test line 1: Two visible colored bands appear at Control line (C) and Test line 1 (T1). Any intensity of Test line indicates the presence of Brazil nut or cashew or pistachio or any combination of these three into the sample.*

Positive Result for Test line 2: Two visible colored bands appear at Control line (C) and Test line 2 (T2). Any intensity of Test line indicates the presence of almond or hazelnut or pecan or walnut or any combination of these four into the sample.

Positive Result for Test line 1 and 2: Three visible colored bands appear at Control line (C), Test line 1 (T1) and Test line 2 (T2). Any intensity of Test lines indicates the presence of any of the seven tree nuts(at least one from each group) into the sample.

Invalid Results: No colored band appears at Control line no matter whether it appears at Test line 1,2 or not.

***NOTE 1:** At a concentration of 10,000 ppm and higher, Test line fades or may not appear for pistachio and Brazil nut (hook effect phenomenon).*



Visual result interpretation index

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NOTE 2: *If you want to determine the level of the allergen you are interested in (quantitative procedure), you can purchase the corresponding product from the* ***Allergen Free*** *series.*

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 a tree nut (or a combination) 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 tree nuts 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 antibodies. Recovery of these matrices might be reduced.
- 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.
- The protein content and the protein composition may differ among various species of the same matrix. Therefore, different varieties may produce different results.

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 for 1min or shake by hand

⇩

Centrifuge the sample for 2 min, at high speed in a microcentrifuge. Alternatively, let the sample settle down

⇩

Transfer 3 drops from the supernatant to the reaction device

⇩

Allow test to develop for 5 minutes

⇩

Read the results visually

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 visually

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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