# AlerTox® Sticks Walnut

Validation Report

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| **Cat No.** | KIT3092 (10 tests) |
| **Test Name** | AlerTox Sticks Walnut |
| **Description** | Immunochromatographic rapid test for qualitative determination of walnut antigen in foods, kitchen and production facilities. |
| **Specificity and Sensitivity** | The LOD (limit of detection) of AlerTox Sticks Walnut is 2.25 ppm of walnut protein. The range of detection (ROD) is 2.25-100,000 ppm of walnut protein. Above this range, the test can present a hook effect.<br><br>AlerTox Sticks Walnut uses a combination of monoclonal antibodies against a major English/common walnut antigen, the 11 S globulin seed storage protein, known as allergen Jug r 4 from *Juglans regia*. Alertox Sticks Walnut does NOT detect the antigens of cereals, legumes and other nuts, including pecan, black walnut, peanut, hazelnut, almond, macadamia, pistachio, cashew, brazil nut and coconut. |
| **Storage** | Store at 10-30 °C (50-86 °F) |

| **Assay Procedure** |
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| Solid, liquid and surface samples: according to manual of use INS3027 |

## Validation Protocol

35 samples were analyzed separately to determine the correlation between AlerTox Sticks Walnut and ELISA against Walnut antigen. The following data was obtained:

| | Sample | Brand | ELISA Test | AlerTox Sticks Walnut |
|---|---|---|---|---|
| 1 | Protein bar with Hazelnut | 012 Nutrition | negative | negative |
| 2 | Protein bar with Almond | 012 Nutrition | negative | negative |
| 3 | Protein cookie coconut | Royal cake | positive | positive |
| 4 | Protein bar with almond | Bombbar | negative | negative |
| 5 | Chocolate bar with walnut | Alpen Gold | positive | positive |
| 6 | Milk chocolate with whole almonds | Milka | negative | negative |
| 7 | Mars | Mars, Inc. | negative | negative |
| 8 | Chocolate bar | Alpen Gold | negative | negative |
| 9 | Geisha chocolate with hazelnut filling | Fazer | negative | negative |
| 10 | Milk Chocolate | Karl Fazer | negative | negative |
| 11 | Milk chocolate with hazelnuts | Dove | negative | negative |
| 12 | Hazelnut drink | ALPRO | negative | negative |
| 13 | Walnut drink | OraSì | positive | positive |
| 14 | Soy drink | ALPRO | negative | negative |
| 15 | Soy calcium drink | NATRUE | negative | negative |
| 16 | Pistachio drink | Simple Foods | negative | negative |
| 17 | Cashew drink | ALPRO | negative | negative |
| 18 | Pistachio almond ice cream | Baskin Robbins | negative | negative |
| 19 | Cheese with nuts | Cheese Lovers | positive | positive |
| 20 | Granola bar with nuts and honey | Everyday | positive | positive |
| 21 | Swiss roll | 7 days | negative | negative |
| 22 | Soft cakes cherry | Fine Life | negative | negative |

| | Sample | Brand | ELISA Test | AlerTox Sticks Walnut |
|---|---|---|---|---|
| **23** | Biscuits filled with lemon cream | Grisbi | negative | negative |
| **24** | Napolitaner original | Manner | negative | negative |
| **25** | Dark chocolate and hazelnut cookies | Merba | negative | negative |
| **26** | Cashew coconut butter | Natty's | negative | negative |
| **27** | Marzipan | ZENTIS | negative | negative |
| **28** | Nutella | Ferrero | negative | negative |
| **29** | Italian bread with black sesame, bran and olive oil | Baker House | negative | negative |
| **30** | Milkshake with the taste of peanut ice cream and nougat | DANONE | negative | negative |
| **31** | Yogurt with nuts, chia seeds and vanilla | DANONE | positive | positive |
| **32** | Dairy dessert with walnuts | Hacendado | positive | positive |
| **33** | Raw walnuts in rice flour | Hygiena reference material | positive | positive |
| **34** | Raw walnut in wheat flour | Hygiena reference material | positive | positive |
| **35** | Raw black walnut | nut.com | positive (low) | negative |

According to these results, the correlation between both techniques is 100% in the samples analyzed.

## Surfaces:

A surface of stainless steel was thoroughly cleaned with water and soap, then rinsed with 60% ethanol, washed again with water and soap and finally rinsed with distilled water and left to dry.

Test areas of 4x4 cm (16cm$^2$) were drawn on the surface and spiked with different levels of walnut extract. The amount of sample was dispensed with a micropipette and distributed throughout the whole surface with the micropipette tip. The areas were left to dry at room temperature, covered to avoid dust.

Spiking samples are prepared from a raw walnut extract and dilutions thereof. Spikings were made at the concentrations shown in the following table (assuming a protein content of 15 g/100 g). Each concentration was tested in duplicate.

| Amount of walnut in extract (ppm) | Amount of walnut in test area (µg/16 cm$^2$) | Approximate amount of walnut protein in test area (µg/16 cm$^2$) | Result |
|---|---|---|---|
| 2500 | 100 | 15 | Positive |
| 2500 | 70 | 10.5 | Positive |
| 2500 | 50 | 7.5 | Positive |
| 1250 | 25 | 3.75 | Positive |
| 625 | 12.5 | 1.9 | Positive |
| 312 | 10 | 1.5 | Negative |
| 312 | 6.25 | 0.9 | Negative |
| 156 | 3.12 | 0.5 | Negative |
| 78 | 2.3 | 0.34 | Negative |
| 39 | 1.56 | 0.23 | Negative |

A faint positive was detected at 1.9 µg/16 cm$^2$ of walnut protein and therefore the limit of detection of the swab protocol was set to 2 µg/16 cm$^2$.