



AgraStrip® Pro Peanut

Part #10007068

Lateral Flow Device (LFD) for qualitative analysis of peanut residues in environmental samples, rinse waters and finished food products.

Cut-off Value:

1 ppm peanut, 1 µg peanut/swab (25 cm²)

Validation Report

Version: 4 (10.2024)

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Log of Changes to the Original method

Last version

Version 3. October 2023

Modifications introduced

Version 3. October 2023

1. Re-validation of the method for a modification introduced in buffer.

Version 2. June 2023

1. Editorial change: document adapted to the new corporate design.

Version 1. March 2023

Original version.

1. Scope

The AgraStrip® Pro Peanut LFD kit is designed for the determination of peanut protein residues in foodstuff and related environmental samples. The present report describes the validation process and its results.

2. Sensitivity

Ground raw peanuts were extracted according to the current product manual. The extract was serially diluted in extraction buffer to different levels to confirm the cut-off value and dynamic range of the test.

Peanut [ppm]	Result
0	-
0.5	+*
1	+
2	+
100	+
100000	+

*very faint lines

The cut-off value of 1 ppm peanut was confirmed in extraction buffer.

Hook effect: the intensity of the test band decreases at 10% peanut but no negative results were registered.

According to the U.S. Department of Agriculture FoodData Central database*, raw peanuts contain 23% protein. Using this value, a theoretical approximation can be made: 1 ppm peanut = 0.2 ppm peanut protein.

IMPORTANT: note that this is a theoretical approximation only. Allergenic materials that have been processed, as well as materials derived from the main allergenic source (e.g., pastes, oils, etc.), might differ greatly in their protein content.

*fdc.nal.usda.gov

3. Specificity

Specificity of the strip was evaluated for a variety of matrices, including grains, nuts and other samples. Samples were prepared in accordance with the current product manual. Extracts of the samples were analysed directly and as a 1:100 dilution.

Sample material	Result	
	Pure	1:100
Almond	-	-
Apricot pit	-	-
Cow's milk	-	-
Hazelnut	+*	-
Hen's egg	-	-
Oat	-	-
Pecan	-	-
Pistachio	+*	-
Rapeseed	-	-
Rice	-	-
Rye	-	-
Soybean	-	-

*very faint line.

Very faint positives were obtained with pure hazelnut and pistachio. Cross-contamination could not be ruled out. Further testing including other types of these nuts will be included in an further investigation to determine cross-reactivity.

4. Application

4.1. Spiked foodstuffs

Sensitivity and specificity of the kits were evaluated for five representative food matrices (cookies, chocolate, curry sauce, margarine, dairy-free margarine, and dairy-free yoghurt). Non-spiked samples were analysed in accordance with the current product manual.

The representative matrices were further analysed with the addition of ground raw peanuts extract at the cut-off level in order to confirm applicability. Spiked samples were mixed well and set aside for at least 1 hour.

Peanut [ppm]	Result					
	Cookie	Chocolate	Curry sauce	Margarine	Yoghurt	Rinse water
0	-	-	-	-	-	-
1	+	+	+	+	+	+
2	+	+	+	+	+	+

The AgraStrip® Pro Peanut LFDs were able to detect 1 ppm peanut in all tested matrices.

Because cacao liquor proved to be a difficult matrix displaying strong interferences with the previous version of the kit, the revalidation of the kit included 4 different cocoa liquors, sourced from different producers and countries. They were analyzed as described above but spiked at a higher cut-off value, since preliminary analysis indicated that this was required for better reproducibility and reliability of the test in this type of matrix.

Peanut [ppm]	Result			
	Cacao liquor A	Cacao liquor B	Cacao liquor C	Cacao liquor D
0	-	-	-	-
2	+	+	+	+

The AgraStrip® Pro Peanut LFDs were able to detect 2 ppm peanut in all tested cocoa liquor samples. Of notice, the control bands obtained for cacao liquor D were less intense than the rest. Centrifugation of the extracts did not impact the results, but allowed for less background coloration of the strips, which might help users read the results more clearly.

4.2. Swabs

The extraction of protein from swabs and detection by the AgraStrip® Pro Peanut LFDs was investigated. Ground raw peanuts were extracted and serially diluted in extraction buffer. The extract dilutions, or extraction buffer alone (blank), were applied to swabs using a micropipette in order to arrive at the allergen amount corresponding to the cut-off level. The swabs were extracted following the instructions in the current product manual.

Peanut [µg]	Result
0	-
0.2	+

The AgraStrip® Pro Peanut LFDs were able to detect 0.2 µg peanut in the swabs. Transfer of allergen from surfaces to the swab remains to be tested. The recommended sampling area is 25 cm².

Since swabbing techniques and efficiency of the allergen collection varies greatly, a nominal cut-off value of 1 µg peanut is set.

5. Robustness

Robustness was determined by varying different handling parameters as defined in the product manual. The results were compared with the results of samples analyzed according to the defined method. A blank and a sample spiked with ground raw peanuts extract at the cut-off level were analyzed respectively.

5.1. Variation of sample size

The mass of sample extracted, defined as 0.2 g, was changed to 0.1 g and 0.3 g, respectively.

Peanut [ppm]	Result		
	0.2 g	0.1 g	0.3 g
0	-	-	-
1	+	+	+

No false positives or false negatives were registered. A change in the intensity of the test bands was noticed, it is recommended to keep the sample size as per protocol.

5.2. Variation of volume of extract

The number of drops of extract added into the incubation vials, defined as 6 drops (200 µL), was changed to 5 drops (approximately 167 µL) and 7 drops (approximately 233 µL), respectively.

Peanut [ppm]	Result		
	6 drops	5 drops	7 drops
0	-	-	-
1	+	+	+

No false positives or false negatives were registered. No change in the intensity of the test bands was noticed.

5.3. Variation of pH of the extract

Given the buffer capacity of the extraction buffer, the pH of the extract is expected to be close to 7. The pH of an extract from a sample of rinse water was measured and confirmed to be neutral (7), and two sub-portions of the same were adjusted to acid (pH, 5) and basic (pH, 9) conditions before incubation.

Peanut [ppm]	Result		
	pH low	pH neutral	pH high
0	-	-	-
1	+	+	+

No false positive or false negative results were registered. No change in the intensity of the test bands was noticed.

5.4. Variation of extract/antibody incubation time

The period the extract is incubated in the incubation vials, defined as 5 min, was extended to 6 min.

Peanut [ppm]	Result	
	5 min	6 min
0	-	-
1	+	+

No false positives or false negatives were registered. No change in the intensity of the test bands was noticed.

5.5. Variation of time of extract flow up the strip

The period the extract is allowed to flow up the strips (lateral flow), defined as 5 min, was extended to 5.5 min (30 sec more).

Peanut [ppm]	Result	
	5 min	5.5 min
0	-	-
1	+	+

No false positives or false negatives were registered. No change in the intensity of the test bands was noticed.

6. Lot-to-lot variability

The performance of the different lots of the kit was evaluated by testing the same blank samples, and samples spiked with ground raw peanuts extract at the cut-off level.

Peanut [ppm]	Result		
	Lot 1	Lot 2	Lot 3
0	-	-	-
1	+	+	+

The performance of all lots was satisfactory. No false positives or false negatives were detected. No difference in the intensity of the test bands was noted between lots.