



AlerTox[®] Sticks Peanut

KIT3094 (10 tests) and KIT3093 (5 tests)

Introduction

AlerTox[®] Sticks Peanut is a rapid, immunochromatographic, lateral flow test for the qualitative detection of peanut antigens in food, kitchens and production facilities. This validation report examines the sensitivity, specificity and cross-reactivity of the AlerTox Sticks Peanut kit on various sample types. Samples included solid, liquid and surface samples and were prepared according to the Product Instructions (IFU), INS-KIT3094.

Summary of Validation Results

Sensitivity, Specificity and Range of Detection

When stored properly (10 to 30 °C (50 to 86 °F)), the following results were obtained.

- The limit of detection (LOD) of AlerTox Sticks Peanut is 1 ppm of raw or roasted peanut protein.
- The range of detection (ROD) is 1 – 250,000 ppm of peanut protein for roasted peanuts; (1 – 150,000 for raw peanuts).
- The LOD of AlerTox Sticks Peanut for surface analysis is approximately 4 µg of roasted peanut protein/ 16 cm² and 2 µg of raw peanut protein/16 cm² on a model, dry surface (stainless steel), sampled with a wet swab.



Validation Protocol

Analytical Sensitivity

Extracted peanut samples

Reference materials (roasted peanut and raw peanut) were extracted according to the kit instructions and the supernatants obtained were serially diluted in extraction buffer. These dilutions, together with negative control samples (extraction buffer alone, or an extracted sample of white rice) were tested with the immunochromatographic strips, according to the kit instructions, with the following results (Table 1):

Table 1. Detection of Peanut Allergen from Extracted Peanut Protein or Whole Peanuts

Reference Material	Comments	Peanut Protein (ppm)	Whole Peanut* (ppm)	Test Result
Raw peanut		1.25	5	Positive
	Limit of detection	1	4	Positive
		0.25	1	Weak Positive
Roasted peanut	Hook effect**	250,000	1,000,000	Weak Positive
		250	1,000	Positive
		100	400	Positive
		25	100	Positive
		1.25	5	Positive
	Limit of detection	1	4	Positive
		0.4	1.6	Weak Positive
	0.25	1	Negative	
Extraction buffer (ASY3209)	Negative control	0	0	Negative
White rice	Negative control	0	0	Negative

* Assuming peanut protein content in peanut = 25 g protein / 100 g of peanut.

** Note: no pure hook effect (= false negative) is seen.

These results (Table 1) indicate that the limit of detection for the AlerTox Stick Peanut test is 1 ppm of peanut protein from raw or roasted peanuts.



Swab Samples

Roasted peanut butter was dispersed in water at different concentrations and 0.045 mL (45 µL) of each sample was applied to a 16 cm² of standard surface material (stainless steel), including a negative control (water) and allowed to dry overnight at room temperature. Surface samples were collected with a wet swab and tested according to the kit instructions, with the following results (Table 2):

Table 2. Detection of Peanut Allergen from Swabbed Surface Samples

Reference material		Peanut Protein on 16 cm ² surface (µg)	Whole Peanut on 16 cm ² surface (µg)	Test Result
Roasted peanut		7	28	Positive
	Limit of detection	3.5	14	Positive
		1.75	7	Negative
Raw peanut		7	28	Positive
		3.5	14	Positive
	Limit of detection	1.75	7	Positive
Water	Negative control	0	0	Negative

The LOD of AlerTox Sticks Peanut for surface analysis is approximately 4 µg of roasted peanut protein/16 cm² surface area and 2 µg of raw peanut protein/16 cm² surface area on a representative, dry surface (stainless steel), sampled with a wet swab (see Table 2 above).

Cross-reactivity and sensitivity in extracts of food commodities

Common food commodities, spiked with 0 (negative control) or 3 ppm of roasted and raw peanut protein (3 times the Claimed Detection Capability, CDC), were extracted according to the kit instructions and each supernatant obtained was tested directly, according to the kit product instructions.

Results are shown in Table 3, which follows.

**Table 3. Detection of Peanut Allergen Cross-reactivity in Other Commodity Samples**

Sample Number	Commodity (Allergen Source)	Results	
		0 ppm Peanut Protein	3 ppm Roasted and Raw Peanut Protein
1	Almond	Negative	Positive
2	Annatto	Negative	Positive
3	Barley	Negative	Positive
4	Brazil nut	Negative	Positive
5	Brown rice	Negative*	Positive
6	Buckwheat	Negative	Positive
7	Cashew	Negative	Positive
8	Corn	Negative	Positive
9	Chestnut	Negative	Positive
10	Cocoa	Negative**	Negative***
11	Chickpea	Negative	Positive
12	Cumin	Negative	Positive
13	Dried coconut	Negative	Positive
14	Fresh Coconut	Positive	Positive
15	Garlic	Negative	Positive
16	Hazelnut	Negative	Positive
17	Heat-treated brown rice	Negative	Positive
18	Kidney beans	Negative	Positive
19	Lentils	Negative	Positive
20	Lima (butter) bean	Negative	Positive
21	Lupine beans	Negative	Positive
22	Macadamia	Negative	Positive
23	Mustard seeds	Negative	Positive
24	Oat	Negative	Positive
25	Paprika	Negative	Positive
26	Pecan	Negative	Positive
27	Pine nut	Negative	Positive
28	Pistachio	Negative	Positive
29	Pumpkin seed	Negative	Positive



Sample Number	Commodity	Results	
	(Allergen Source)	0 ppm Peanut Protein	3 ppm Roasted and Raw Peanut Protein
30	Red beans	Negative	Positive
31	Rye	Negative	Positive
32	Sesame seed	Negative	Positive
33	Soy flour	Negative	Positive
34	Sunflower seed	Negative	Positive
35	Walnut	Negative	Positive
36	Wheat	Negative	Positive
37	White rice	Negative	Positive
38	Ginger	Negative	Positive at 7.5 ppm [†]
39	Pine nut roasted	Negative	Positive at 5 ppm [†]
40	Thyme	Negative	Positive at 5 ppm [†]
41	Turmeric	Negative	Negative at 10 ppm [†]

* Some commercially available raw brown rice could produce a positive result that is eliminated with heat treatment of the sample.

** Further diluted to 90,000 ppm in extraction buffer, to reduce excessive extract thickness before testing.

*** False negative due to matrix interference. See specific protocol and validation for cocoa liquor and chocolate.

† Tested with roasted peanut protein.

Validation with Commercial Food Products

Selection of Common Commercial Food Products

The following commercially available products were analyzed to determine the correlation between AlerTox Sticks Peanuts and the food labeling claim. Results are in Table 4.

Table 4. Identification of Peanut Allergen in Commercial Food Products

Sample Number	Commodity	Brand	Contains/May contain (according to food labeling)	Result LFD
1	Coconut yogurt	Danone	Contains milk	Negative
2	Coconut macaroon	Mas	Contains shredded coconut	Negative
3	Coconut milk	Dee Thai	Contains coconut extract and coconut milk	Negative
4	Grated coconut	Hacendado	Dehydrated shredded coconut	Negative
5	Coconut bonbon	Raffaello	Milk, soy, coconut, gluten, almond	Negative
6	Coconut nougat	Hacendado	Sulfites, nuts (almond, hazelnut, walnut, pistachio), egg and soy.	Negative
7	Coconut protein bar	Lidl	Nuts and egg	Negative



Sample Number	Commodity	Brand	Contains/May contain (according to food labeling)	Result LFD
8	Lemon Juice	N/A	Lemon	Positive
9	Neutralized lemon juice	N/A	6N NaOH added	Negative
10	Brown rice milk	Isola Bio	N/A	Negative
11	Cocoa peanut bar	Snickers	Contains peanut , milk, soy, egg. May contain nuts.	Positive
12	Integral oat flour	Harimsa	Gluten, milk, soy, egg	Negative
13	Sesame oil	KTC	Sesame	Negative
14	Gluten free María cookie	Gullón	Soy	Negative
15	Teriyaki sauce	Sauce shop	Soybeans, sesame	Negative
16	Chocolate candy	Lacasitos	Peanuts , nuts, soy	Positive
17	Chocolate bar	Mars	Contains milk, soy egg. May contains peanut and hazelnut	Negative
18	Peanut and cocoa candy	M&M'S	Peanuts . May contains nuts, soy, gluten	Positive
19	Cookie	Lotus Biscoff	Soy, gluten	Negative
20	Milk chocolate and hazelnut rice cake	Bicentury	Milk, soy, hazelnut, nuts, gluten	Negative
21	Cheese breadsticks	Eliges	Milk, gluten. May contains peanut and sesame	Negative
22	Dark chocolate-covered corn cakes	Gullón	Soy, milk	Negative
23	Brown rice Bio	Ecocesta Bio (ecological agriculture)	Gluten, soy, sesame, nuts	Positive
24	Cooked Brown rice Bio	Ecocesta Bio (ecological agriculture)	Gluten, soy, sesame, nuts	Negative
25	Brown rice	Hacendado (Arrocierias Pons SAU)	N/A	Negative

Test of Cocoa Liquor and Cocoa Powder

Samples of cocoa liquor (liquid cocoa) and cocoa/chocolate powder, spiked with different amounts of raw or roasted peanut protein were extracted according to the kit instructions, in the absence or the presence of the recommended additive ASY3213 (AlerTox Polyphenol Extraction Additive). The use of the additive reduced the interfering effect of the matrix, resulting in LOD values of 1 ppm or 4 ppm of protein from raw and roasted peanut, respectively for cocoa liquor (Table 5) and 10 ppm or 15 ppm of protein from raw and roasted peanut, respectively for cocoa/chocolate powder (Table 6). Results are shown in Table 5 below.

**Table 5. Detection of Peanut Allergen in Cocoa Liquor (46.5% non-fat cocoa and 53.5% cocoa butter)**

Spiked Level of Peanut Protein	Additive (ASY3213)	Comment	Result
0	-		Negative
0	1 spoonful		Negative
25 ppm roasted peanut protein	-		Positive
15 ppm roasted peanut protein			Positive
10 ppm roasted peanut protein			Negative
4 ppm roasted peanut protein	1 spoonful	Limit of detection	Positive
25 ppm raw peanut protein	-		Positive
10 ppm raw peanut protein	-		Positive
1 ppm raw peanut protein	1 spoonful	Limit of detection	Positive

**Table 6. Detection of Peanut Allergen in Chocolate*
(70% cocoa paste, sugar, fat-reduced cocoa powder, cocoa butter, emulsifier, lecithin (soya))**

Spiking level	Additive (ASY3213)	Comment	Result
0	-		Negative
0	1 spoonful		Negative
20 ppm roasted peanut protein	1 spoonful		Positive
15 ppm roasted peanut protein	1 spoonful	Limit of detection	Positive
10 ppm roasted peanut protein	1 spoonful		Negative
5 ppm roasted peanut protein	1 spoonful		Negative
10 ppm raw peanut protein	1 spoonful	Limit of detection	Positive
5 ppm raw peanut protein	1 spoonful		Negative

* **Note:** Please contact your provider for limit of detection validations of specific chocolate composition.

Conclusion

Based on the results of various sample and commodity testing, the LOD (limit of detection) of AlerTox Sticks Peanut is 1 ppm of raw or roasted peanut protein. For roasted peanuts, the range of detection (ROD) is 1 – 250,000 ppm of peanut protein. Overloading (signal decrease) may be seen at 2,500 – 250,000 ppm; however, no pure hook effect (false negative) is observed.

For raw peanuts, the ROD is 1 – 150,000 ppm of peanut protein. Overloading (signal decrease) may be seen at 500 – 150,000 ppm. Above 150,000 ppm, a hook effect appears as a negative result (false negative). If a false negative due to the hook effect is suspected, the testing should be repeated using a diluted sample.

The LOD of AlerTox Sticks Peanut for surface analysis is approximately 4 µg of roasted peanut protein/16 cm² and 2 µg of raw peanut protein/16 cm² on a representative, dry surface (stainless steel), sampled with a wet swab.