AlerTox® ELISA Almond Kit

Claim Support Summary

REF KIT3049

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1. Scope

The AlerTox® ELISA Almond Kit is based on a polyclonal antibody and designed to determine almond content in food. This report describes the validation process and its results.

Definitions

CV = Coefficient of variation

IQR = Interquartile range

LOD = Limit of detection

LOQ = Limit of quantification

OD = Optical density

ppm = parts per million = mg of almond protein per kg or L of sample (mg/kg or mg/L)

PT = Proficiency testing

SD = Standard deviation

2. Precision

2.1 Intra-Assay Variation

The intra-assay variation (repeatability) was determined by testing a control at 3 concentrations (0.4, 1 and 8 ppm) in 24-fold replicates, using either sample diluent (Table 1) or a cookie matrix (Table 2). After identification of possible outliers, the following results were obtained:

Table 1. AlerTox ELISA Almond Kit Intra-Assay Variation with Sample Diluent.

Replicate	0.4 ppm	1 ppm	8 ppm	
1	0.32	1.00	8.27	
2	0.33	1.06	8.80	
3	0.36	0.96	8.84	
4	0.35	1.04	8.67	
5	0.37	0.99	8.60	
6	0.37	0.98	8.35	
7	0.39	0.96	7.38	
8	0.37	0.99	8.11	
9	0.37	0.99	8.72	
10	0.43	0.97	8.12	
11	0.39	0.97	7.97	
12	0.41	0.98	8.44	
13	0.44	0.97	7.39	
14	0.44	0.98	8.08	
15	0.37	1.01	8.03	
16	0.39	1.02	8.53	
17	0.43	0.96	7.89	
18	0.44	1.06	7.33	
19	0.44	1.07	7.52	
20	0.41	1.07	7.93	
21	0.32	1.04	7.70	
22	0.37	1.06	8.64	
23	0.41	0.99	8.04	
24	0.39	1.00	7.02	
Mean	0.39	1.00	8.10	
SD	0.04	0.04	0.51	Mean
CV [%]	**9.4**	**3.8**	**6.3**	**6.5**

The coefficient of variation (CV) with sample diluent ranged from 3.8 to 9.4%, depending on the concentration tested.

Table 2. AlerTox ELISA Almond Kit Intra-Assay Variation with a Cookie Matrix.

Replicate	0.4 ppm	1 ppm	8 ppm	
1	0.43	0.96	8.2	
2	0.49	0.92	8.3	
3	0.47	0.99	8.3	
4	0.56	0.92	7.7	
5	0.52	0.99	9.0	
6	0.54	0.88	8.9	
7	0.49	0.92	8.8	
8	0.45	0.96	8.3	
9	0.44	0.97	7.7	
10	0.50	0.95	8.5	
11	0.47	0.99	8.0	
12	0.56	0.93	7.9	
13	0.51	0.94	8.8	
14	0.47	0.95	8.6	
15	0.44	0.92	8.2	
16	0.46	0.95	7.9	
17	0.47	0.98	7.9	
18	0.55	1.00	8.5	
19	0.53	0.95	8.9	
20	0.51	0.92	9.0	
21	0.46	0.86	8.7	
22	0.47	0.86	8.3	
23	0.49	0.88	8.1	
24	0.52	0.95	8.7	
Mean	0.49	0.94	8.4	
SD	0.04	0.04	0.41	Mean
CV [%]	**8.0**	**4.1**	**4.9**	**5.7**

The CV with a cookie matrix ranged from 4.1 to 8.0%, depending on the concentration tested.

2.2 Inter-Assay Variation

The inter-assay variation (intermediate precision) was determined by testing a control at 3 concentrations (0.4, 1 and 8 ppm) in 4 different test runs of the same kit lot, using either sample diluent (Table 3) or a cookie matrix (Table 4).

Table 3. AlerTox ELISA Almond Kit Inter-Assay Variation with Sample Diluent.

Assay Number	0.4 ppm	1 ppm	8 ppm	
1	0.38	0.99	8.98	
2	0.43	1.05	8.64	
3	0.48	0.92	8.10	
4	0.49	0.90	8.34	
Mean	0.44	0.96	8.51	
SD	0.05	0.07	0.38	Mean
CV [%]	**10.6**	**6.9**	**4.5**	**7.3**

The CV with sample diluent ranged from 4.5 to 10.6%, depending on the concentration tested.

Table 4. AlerTox ELISA Almond Kit Inter-Assay Variation with a Cookie Matrix.

Assay Number	0.4 ppm	1 ppm	8 ppm	
1	0.40	0.95	8.1	
2	0.38	1.01	8.1	
3	0.35	0.99	8.8	
4	0.41	0.97	8.8	
Mean	0.39	0.98	8.5	
SD	0.03	0.02	0.41	Mean
CV [%]	**6.7**	**2.4**	**4.8**	**4.7**

The CV with a cookie matrix ranged from 2.4 to 6.7%, depending on the concentration tested.

2.3 Reproducibility of Extraction

To determine the reproducibility of the extraction process, an almond proficiency test (PT) material (DLA 14/2019, sample 1) was analyzed. The PT material provider assessed the material's homogeneity by analyzing 20 different samples with 3 different test kits. According to the PT report, the micro tracer analysis of the material resulted in a probability of 85%.

The material was extracted 10 times, and the CV was calculated using the mean results from all extracts (Table 5).

Table 5. Reproducibility of Extraction of the AlerTox ELISA Almond Kit.

Extraction Number	Mean Result [ppm]
1	33.2
2	37.1
3	32.4
4	30.4
5	38.5
6	32.7
7	33.2
8	34.1
9	32.2
10	35.1
Mean	33.9
SD	2.42
CV [%]	**7.2**

The CV was 7.2 for the inter-extraction reproducibility results.

3. Analytical Sensitivity

To determine the analytical sensitivity of the AlerTox ELISA Almond Kit, sample diluent and various representative, almond-free sample matrices were assayed in 24-fold replicates. After identification of possible outliers, the mean and standard deviation of the optical density (OD) were calculated. The corresponding concentration of the mean OD + 3X standard deviation (SD) was defined as the limit of detection (LOD) (Tables 6a and 6b).

Note: In some cases. various samples of the same kind (e.g., different cookies) were tested. Table 6a represents the matrix with the highest LOD, while Table 6b summarizes the results.

Table 6a. Matrix-Independent and Matrix-Dependent Analytical Sensitivity of the AlerTox ELISA Almond Kit (Complete Data).

Replicate	Sample Diluent [OD]	Cookie Matrix [OD]	Sweets Matrix [OD]	Ice Cream Matrix [OD]	Chocolate Matrix* [OD]	Muesli Matrix [OD]	Food Supplement Matrix [OD]
1	0.033	0.061	0.075	0.059	0.128	0.043	0.025
2	0.036	0.064	0.082	0.066	0.128	0.042	0.026
3	0.038	0.068	0.068	0.063	0.128	0.045	0.024
4	0.040	0.051	0.085	0.051	0.120	0.041	0.021
5	0.041	0.059	0.066	0.066	0.118	0.039	0.023
6	0.046	0.063	0.058	0.051	0.130	0.042	0.028
7	0.034	0.060	0.075	0.050	0.123	0.049	0.028
8	0.048	0.053	0.063	0.048	0.119	0.035	0.033
9	0.040	0.054	0.060	0.053	0.124	0.043	0.022
10	0.034	0.051	0.075	0.043	0.124	0.046	0.024
11	0.034	0.055	0.067	0.045	0.112	0.041	0.019
12	0.047	0.058	0.075	0.056	0.131	0.035	0.020
13	0.036	0.060	0.059	0.043	0.115	—	0.019
14	0.041	0.060	0.068	0.048	0.125	0.042	0.023
15	0.038	0.057	0.073	0.042	0.123	0.040	0.031
16	0.045	0.056	0.080	0.046	0.135	0.036	0.030
17	0.037	0.058	0.059	0.055	0.128	0.039	0.020
18	0.040	0.062	0.083	0.045	0.120	0.036	0.017
19	0.043	0.051	0.062	0.048	0.116	0.050	0.019
20	0.043	0.052	0.085	0.069	0.130	0.042	0.022
21	0.046	0.048	0.078	0.044	0.115	0.040	0.019
22	0.046	0.048	0.084	0.057	0.117	0.035	0.022
23	0.033	0.048	0.070	0.055	0.121	0.036	0.026
24	0.037	0.060	0.079	0.047	0.129	0.041	0.025
Mean	0.040	0.057	0.072	0.052	0.123	0.041	0.024
SD	0.005	0.005	0.009	0.008	0.006	0.005	0.004
LOD [ppm]	**0.06**	**0.07**	**0.06**	**0.02**	**0.20**	**< 0.005**	**< 0.005**
Corresponding Calibration Curve Data [OD]							
0 ppm	0.040	0.046	0.069	0.068	0.055	0.052	0.039
0.4 ppm	0.144	0.195	0.260	0.233	0.229	0.262	0.170
1 ppm	0.332	0.350	0.532	0.459	0.467	0.414	0.363
4 ppm	1.037	1.148	1.482	1.364	1.498	1.410	1.038
10 ppm	1.910	2.005	2.692	2.387	2.455	2.536	1.934

* Used the AlerTox Polyphenol Additive during sample extraction.

—= data not used.

Table 6b. Matrix-Independent and Matrix-Dependent Analytical Sensitivity of the AlerTox ELISA Almond Kit (Summary).

Matrix Type	Variant	LOD (ppm)
Sample diluent	N/A	0.06
Cookies	Butter cookie	0.07
	Oat-spelt cookie	0.05
	TUC cookie	0.03
Sweets	Type 1	0.01
	Candy bar	0.06
	Vitamin drop	0.01
Ice cream	Type 1	0.08
	Type 2	< 0.005
Chocolate*	Yogurt cubes	0.05
	Sarotti, 72% cocoa	0.20
	Lindt, 70% cocoa	0.05
Muesli	Type 1	< 0.005
Food supplement	Type 1	0.01
	Almased	< 0.005
	Wellmix, Classic	< 0.005

* Used the AlerTox Polyphenol Additive during sample extraction.

LODs vary from < 0.005 to 0.20 ppm, depending on the sample matrix. Note that the derived LODs are strictly dependent on the CV and thus may vary in individual tests.

The lowest positive standard (0.5 ppm) provided in the kit was defined as the limit of quantification (LOQ) to ensure that measurements of all uncontaminated matrices result in concentrations lower than this value.

4. Recovery

4.1 Food Samples

For recovery experiments, different sample matrices were spiked with an almond extract at 3 final concentrations (0.4, 1 and 8 ppm) after performing all sample pre-treatment steps. The result of the unspiked matrix was subtracted before evaluation. The tested samples and calculated results are shown in Table 7.

Table 7. Recovery of Various Samples Tested with the AlerTox ELISA Almond Kit.

Cookie: Butter Cookie **Unspiked Matrix: 0.05 ppm**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
0.4 ppm	0.45	113
1 ppm	1.02	102
8 ppm	8.11	101
	Mean	106

Cookie: Oat-Spelt Cookie **Unspiked Matrix: 0.04 ppm**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
0.4 ppm	1.13	113
1 ppm	4.16	104
8 ppm	19.7	98
	Mean	105

Cookie: TUC Cookie **Unspiked Matrix: 0.03 ppm**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
0.4 ppm	0.37	94
1 ppm	0.92	92
8 ppm	8.08	101
	Mean	96

Muesli **Unspiked Matrix: 0.00 ppm**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
0.4 ppm	0.48	119
1 ppm	1.24	124
8 ppm	8.31	104
	Mean	115

Sweets: Candy Bar **Unspiked Matrix: 0.00 ppm**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
0.4 ppm	0.43	108
1 ppm	0.93	93
8 ppm	7.92	99
	Mean	100

Sweets: Vitamin Drop* **Unspiked Matrix: 0.00 ppm**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
0.4 ppm	0.38	95
1 ppm	0.95	95
8 ppm	9.19	115
	Mean	102

Sweets: Yogurt Cubes* **Unspiked Matrix: 0.00 ppm**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
0.4 ppm	0.32	80
1 ppm	1.02	102
8 ppm	6.58	82
	Mean	88

* Used the AlerTox Polyphenol Additive during sample extraction.

Chocolate: Sarotti (72% Cocoa)* **Unspiked Matrix: 0.19 ppm**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
0.4 ppm	0.30	76
1 ppm	1.34	134
8 ppm	8.46	106
	Mean	105

Chocolate: Lindt (70% Cocoa)* **Unspiked Matrix: 0.00 ppm**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
0.4 ppm	0.38	96
1 ppm	0.95	95
8 ppm	7.73	97
	Mean	96

Chocolate: Type 1* **Unspiked Matrix: 0.00 ppm**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
0.4 ppm	0.29	71
1 ppm	0.72	72
8 ppm	5.68	71
	Mean	71

Food Supplement: Almased **Unspiked Matrix: 0.00 ppm**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
0.4 ppm	0.47	118
1 ppm	1.04	104
8 ppm	8.31	104
	Mean	109

Food Supplement: Wellmix, Classic **Unspiked Matrix: 0.00 ppm**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
0.4 ppm	0.27	67
1 ppm	0.73	73
8 ppm	10.21	128
	Mean	89

Food Supplement: Wellmix, Vanilla **Unspiked Matrix: 0.00 ppm**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
0.4 ppm	0.32	79
1 ppm	0.70	70
8 ppm	5.43	68
	Mean	72

* Used the AlerTox Polyphenol Additive during sample extraction.

Ice Cream: Yogurt	Unspiked Matrix: 0.00 ppm	
Spiked Value	**Measured Concentration [ppm]**	**Recovery [%]**
0.4 ppm	0.25	63
1 ppm	0.73	73
8 ppm	6.47	81
	Mean	72

Ice Cream: Solero	Unspiked Matrix: 0.00 ppm	
Spiked Value	**Measured Concentration [ppm]**	**Recovery [%]**
0.4 ppm	0.30	76
1 ppm	0.66	66
8 ppm	7.35	92
	Mean	78

Mean recoveries ranged from 72 to 115%, depending on the sample matrix.

4.2 Heat-treated Matrices

To assess potential changes in the analytical sensitivity and recovery of processed food samples, baked or unbaked cookie matrices with or without almonds were analyzed. The spiked cookie matrix had 10 ppm of almond and was serially diluted 1:2 to 1.25 ppm. Each unspiked or spiked sample was then divided into 2 parts that were either left unbaked or baked at 150 °C for 20 min (Table 8). All tests were performed in duplicates.

Table 8. Accuracy of the AlerTox ELISA Almond Kit with Heated-treated Matrices.

Matrix	No Almond Spike [0 ppm]	Almond Spike [1.25 ppm]	Almond Spike [2.5 ppm]	Almond Spike [5 ppm]	Almond Spike [10 ppm]
Unbaked cookie	0.03	0.95	2.33	4.58	9.28
Baked cookie	0.00	0.92	2.21	4.48	9.07

No significant difference between the baked and unbaked cookie samples was observed.

4.3 Rinse and Clean-in-Place (CIP) Water

The AlerTox ELISA Almond Kit sensitivity with rinse and CIP water samples was determined by testing various concentrations of diluted almond extract in different commonly used, water-based rinse solutions. The pH was adjusted to 8.2 ± 0.5 before running the test. Afterwards, the samples were diluted 1:5 in pre-diluted sample extraction buffer. The samples tested and calculated results are shown in Table 9.

Table 9. Recovery with Rinse Solution Samples Tested Using the AlerTox ELISA Almond Kit.

Pure Water **Unspiked Matrix: 0.01 mg/L**

Spiked Value	Measured Concentration [mg/L]	Recovery [%]
0.1 mg/L	0.32	79
0.2 mg/L	0.76	95
0.4 mg/L	1.29	80
0.8 mg/L	2.76	86
1.6 mg/L	6.29	98
	Mean	88

0.5% Sodium Triphosphate **Unspiked Matrix: 0.06 mg/L**

Spiked Value	Measured Concentration [mg/L]	Recovery [%]
0.1 mg/L	0.35	87
0.2 mg/L	0.76	95
0.4 mg/L	1.27	79
0.8 mg/L	2.08	65
1.6 mg/L	5.50	86
	Mean	82

2% Sodium Hydroxide **Unspiked Matrix: 0.01 mg/L**

Spiked Value	Measured Concentration [mg/L]	Recovery [%]
0.1 mg/L	0.46	115
0.2 mg/L	1.01	126
0.4 mg/L	1.71	107
0.8 mg/L	2.78	87
1.6 mg/L	5.35	84
	Mean	104

0.5% Nitric Acid **Unspiked Matrix: 0.00 mg/L**

Spiked Value	Measured Concentration [mg/L]	Recovery [%]
0.1 mg/L	0.33	83
0.2 mg/L	0.88	109
0.4 mg/L	1.87	117
0.8 mg/L	2.84	89
1.6 mg/L	5.93	93
	Mean	98

0.5% Citric Acid	Unspiked Matrix: 0.02 mg/L	
Spiked Value	**Measured Concentration [mg/L]**	**Recovery [%]**
0.1 mg/L	0.46	114
0.2 mg/L	0.75	94
0.4 mg/L	1.89	118
0.8 mg/L	2.58	81
1.6 mg/L	5.65	88
	Mean	99

Mean recoveries for rinse water ranged from 82 to 104%, depending on the kind of rinse solution.

5. Linearity

Linearity was determined by spiking various matrices with an almond extract and testing serial dilutions (10 to 0.625 ppm) of the resulting matrices (Table 11). For calculating linearity, the highest concentration was defined as the reference value (100%), and further dilutions were expressed as a percentage of this reference after accounting for the dilution factor. For the matrices extracted with the addition of the AlerTox Polyphenol Additive, further dilution was done with the standard extraction buffer that did not include the additive.

Table 11. Matrix-Dependent Linearity of the AlerTox ELISA Almond Kit.

Cookie: Butter Cookie

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	10.3	Reference* (100)
5 ppm	4.8	100
2.5 ppm	2.44	96
1.25 ppm	1.29	90
0.625 ppm	0.62	101
	Mean	97

Muesli

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	10.7	Reference* (100)
5 ppm	5.6	105
2.5 ppm	2.45	91
1.25 ppm	1.30	97
0.625 ppm	0.66	99
	Mean	98

* The highest concentration was defined as the reference value (100%). Further dilutions were expressed as a percentage of this reference after accounting for the dilution factor.

Chocolate: Lindt (70% Cocoa)†

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	9.6	Reference* (100)
5 ppm	4.8	101
2.5 ppm	2.46	102
1.25 ppm	1.18	98
0.625 ppm	0.60	100
	Mean	100

Food Supplement: Almased

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	9.3	Reference* (100)
5 ppm	4.6	100
2.5 ppm	2.28	98
1.25 ppm	1.07	93
0.625 ppm	0.54	93
	Mean	96

Ice Cream: Solero

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	8.7	Reference* (100)
5 ppm	4.3	99
2.5 ppm	2.19	101
1.25 ppm	1.00	92
0.625 ppm	0.57	105
	Mean	99

Sweets: Vitamin Drop†

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	10.0	Reference* (100)
5 ppm	4.6	91
2.5 ppm	2.30	92
1.25 ppm	1.16	93
0.625 ppm	0.62	98
	Mean	93

* The highest concentration was defined as the reference value (100%). Further dilutions were expressed as a percentage of this reference after accounting for the dilution factor.

† Used the AlerTox Polyphenol Additive during sample extraction.

For different matrices, the mean linearity ranged from 93 to 100%. The linearity was independent of the specific almond concentration and may have been affected by the precision, as stated in Section 2.

6. Cross-Reactivity

No cross-reactivity is defined as a result that is less than the LOQ. For the AlerTox ELISA Almond Kit, the LOQ is 0.5 ppm whole almond. During testing, no cross-reactivity was detected in the foods included in Table 12.

Table 12. Non-Cross-Reactive Food Matrices with the AlerTox ELISA Almond Kit.

Non-Cross-Reactive Matrices				
Adzuki bean	Apricot	Barley	Bean, white	Beef
Brazil nut	Buckwheat	Cabbage, white	Cardamom	Carob gum
Carrot	Cashew	Cayenne	Celery	Cherry*
Chestnut	Chicken	Chickpea	Chili	Coconut
Cod	Corn	Cumin*	Dill	Duck
Egg (dried)	Fennel	Fenugreek	Flaxseed	Garden cress
Garlic (fresh)	Garlic (granule)	Gelatin, cow	Ginger (fresh)	Gliadin
Guar gum	Gum arabic	Hazelnut	Kidney bean*	Kiwi
Lamb	Leek	Lentil*	Lupine	Milk, cow
Milk, goat	Mustard, yellow	Mustard, black	Nutmeg	Oats
Onion	Paprika	Pea	Peanut	Peach
Pecan	Pepper, black	Pine seed	Pistachio	Pork
Potato	Prawn	Pumpkin seed	Rapeseed	Rice
Rye	Saccharose	Sesame	Shrimps	Soy flour
Soy lecithin	Split pea	Sunflower seed	Thyme*	Tofu
Tomato	Turkey	Turmeric	Walnut	Wheat

* Cherry, cumin, kidney bean, lentil and thyme showed results between 0.5 LOQ and 1 LOQ and may provide values above the LOQ.

The following cross-reactivities were determined (Table 13):

Table 13. Cross-Reactive Food Matrices with the AlerTox ELISA Almond Kit.

Cross-Reactive Food Matrices	
Raw Material	Cross-reactivity [%]
Apricot kernel	100
Peach kernel	16
Cherry kernel	1.7
Mahaleb cherry kernel	1.4
Plum kernel	1
Clove	0.0002
Chia	0.0002
Cocoa	0.0001
Poppy seed	0.0001
Radish	0.00008
Horseradish	0.00007
Macadamia	0.00007
Cinnamon	0.00006
Caraway	0.00004
Ginger (ground)	0.00004
Sweet red pepper seeds	0.00004

7. Biological or Process Variants

Industrial processing (e.g., heating, pH changes, fermentation) could result in structural variants of almond allergens that differ from the target allergen detected by the AlerTox ELISA Almond Kit. To assess the reactivity towards other variants, unroasted samples were compared to samples roasted at 150 °C for varying amounts of time (Table 14).

Table 14. Accuracy (Relevant Analyte Variants) of the AlerTox ELISA Almond Kit.

	Not Roasted	Roasted at 150 °C			
		15 min	30 min	60 min	120 min
Reactivity [%]	**100**	94	86	38	25

Variant-dependent reactivity ranged from 25 to 100%.

8. Robustness

Robustness was determined by the variation of different handling parameters defined in the instruction manual. The results obtained under various conditions were compared to the results obtained by following the instruction manual. An unspiked cookie sample and a sample spiked with almond extract were analyzed.

8.1 Variation of Extraction Temperature

The extraction temperature, defined as 60 °C, was changed to 25, 40 and 70 °C (Table 15).

Table 15. Variation of Extraction Temperature with the AlerTox ELISA Almond Kit.

Sample	Result 60 °C	Result 25 °C	Result 40 °C	Result 70 °C
Cookie, 0 ppm almond	**< 0.4 ppm**	< 0.4 ppm	< 0.4 ppm	< 0.4 ppm
Cookie, 4 ppm almond	**3.99 ppm**	4.17 ppm	4.15 ppm	4.27 ppm

Considering the precision data (Section 2), the results did *not* differ significantly.

8.2 Variation of Extraction Time

The extraction time, defined as 15 min, was changed to 10 and 20 min (Table 16).

Table 16. Variation of Extraction Time with the AlerTox ELISA Almond Kit.

Sample	Result 15 min	Result 10 min	Result 20 min
Cookie, 0 ppm almond	**< 0.4 ppm**	< 0.4 ppm	< 0.4 ppm
Cookie, 4 ppm almond	**3.72 ppm**	4.10 ppm	4.22 ppm

Considering the precision data (Section 2), the results did *not* differ significantly.

8.3 Drift

In contrast to the test procedure defined in the instruction manual, the incubation time of the samples was extended and reduced by 4 minutes compared to the calibrators (20 min) (Table 17).

Table 17. Drift in the AlerTox ELISA Almond Kit.

Sample	Result 20 min	Result 16 min	Result 24 min
Cookie, 0 ppm almond	**< 0.4 ppm**	< 0.4 ppm	< 0.4 ppm
Cookie, 4 ppm almond	**3.60 ppm**	3.07 ppm	4.25 ppm

The results differed significantly. Drift in extensive test runs should be avoided by pipetting calibrators once before and once after the samples and using the mean value for the calculation.

9. Summary

- The AlerTox ELISA Almond Kit is sensitive with an LOD of 0.2 ppm (mg of whole almond per kg or L of sample), which is the upper limit of the matrices tested. The LOQ is 0.5 ppm whole almond (mg/kg or mg/L), based on the lowest positive standard provided in the kit.
- The AlerTox ELISA Almond Kit provides consistent, precise results, as indicated by results of the intra- and inter-assay studies (mean CV < 10%, Section 2).
- Robust recovery (Section 4) and linearity (Section 5) mean that samples and their dilutions are quantitated accurately.
- Extensive cross-reactivity studies showed high specificity (Section 6) except for apricot and peach kernel (100 and 16% cross-reactivity, respectively). A few other matrices (n = 14) showed cross-reactivity of ≤ 1.7%
- **Important**: Drift in large test runs can be avoided by pipetting calibrators once before and once after the samples and using the calibrator mean values for the calculations.