Validation Report

AlerTox ELISA Almond

KIT3049/KT-5910

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1. Scope

The AlerTox ELISA Almond is designed for the determination of almond in food. The present report describes the validation process and its results.

2. Precision

A) Intra-Assay Variation

The intra-assay variation was determined by testing three controls of various concentration levels in 20fold replicates.

Table 1: Intra-assay variation of the AlerTox ELISA Almond

Replicate	Level 1	Level 2	Level 3	
1	1.10	3.62	8.47	
2	1.07	3.72	8.71	
3	0.98	3.75	9.13	
4	0.93	3.43	9.26	
5	0.88	3.62	8.95	
6	0.91	3.65	9.03	
7	0.91	3.54	9.30	
8	0.81	3.72	9.30	
9	0.96	3.63	8.25	
10	0.90	3.49	9.82	
11	0.88	3.60	9.66	
12	0.84	3.49	9.48	
13	0.83	3.68	9.72	
14	0.90	3.75	9.80	
15	0.89	3.80	9.04	
16	0.79	3.55	8.48	
17	1.00	3.65	9.16	
18	0.93	3.67	8.96	
19	0.89	3.65	9.06	
20	0.84	3.46	9.22	
Mean	0.91	3.62	9.14	
SD	0.08	0.10	0.44	**RMS**
CV [%]	**8.8**	**2.9**	**4.8**	**6.0**

The coefficient of variation is ranging from 2.9% to 8.8% depending on the concentration.

RMS = Root Mean Square

B) Inter-Assay Variation

The inter-assay variation was determined by testing three controls of various concentration levels in four different test runs of the same kit lot.

Table 2: Inter-assay variation of the AlerTox ELISA Almond

Assay No.	Level 1	Level 2	Level 3	
1	0.92	3.35	8.74	
2	0.93	3.47	7.32	
3	0.70	3.24	7.47	
4	0.95	3.46	8.29	
Mean	0.88	3.38	7.95	
SD	0.12	3.35	8.74	**RMS**
CV [%]	**13.4**	**3.2**	**8.5**	**9.4**

The coefficient of variation is ranging from 3.2% to 13.4% depending on the concentration.

3. Recovery

For recovery experiments different sample matrices were spiked with almond to obtain various final concentrations after performing all sample pre-treatment steps. Tested samples and results were as follows.

Table 3: Recovery of various samples tested with the AlerTox ELISA Almond

Cornflakes

Target Value	Actual Concentration	Recovery [%]
1 ppm	1.13	113
4 ppm	4.01	100
	Mean	**107**

Cookies

Target Value	Actual Concentration	Recovery [%]
1 ppm	0.91	91
4 ppm	3.62	91
	Mean	**91**

Dark chocolate

Target Value	Actual Concentration	Recovery [%]
1 ppm	0.59	59
4 ppm	3.19	80
	Mean	**69**

Ice-cream

Target Value	Actual Concentration	Recovery [%]
1 ppm	0.81	81
4 ppm	3.07	77
	Mean	**79**

Mean recoveries are ranging from 69% to 107% depending on the sample matrix.

4. Analytical Sensitivity

For determination of the analytical sensitivity sample diluent was assayed in 24fold replicates. After identification of possible outliers, the OD mean and standard deviation was calculated. The corresponding concentration of the OD_{mean} + 3x standard deviation was defined as limit of detection.
This results in limits of detection according to the following table:

Table 4: Matrix-independent analytical sensitivity of the AlerTox ELISA Almond

Replicate	Sample diluent [OD]
1	0.094
2	0.086
3	0.093
4	0.092
5	0.093
6	0.098
7	0.141
8	0.094
9	0.088
10	0.089
11	0.105
12	0.089

Replicate	Sample diluent [OD]
13	0.086
14	0.086
15	0.095
16	0.091
17	0.087
18	0.086
19	0.081
20	0.085
21	0.085
22	0.100
23	0.126
24	0.124
Mean	**0.096**
SD	**0.015**
Limit of Detection	**0.2 ppm**

The limit of detection is 0.2 ppm of almond. The lowest positive standard (0.5 ppm) was defined as limit of quantification (LOQ).

5. Linearity

Linearity was determined by spiking cookies, chocolate, cornflakes and ice- cream samples with almond and testing subsequent dilutions of the resulting extracts. For calculation of the linearity the highest concentration was defined as reference value (100%) and further dilutions were expressed in per cent of this reference after consideration of the dilution factor.

Table 5: Matrix dependent linearity of the AlerTox ELISA Almond

Cookies

Target Value	Concentration [ppm]	Recovery [%]
10 ppm	9.26	100
5 ppm	4.54	98
2.5 ppm	2.08	90
1.25 ppm	0.89	77
0.63 ppm	0.51	88
	Mean [%]	**88**

Dark Chocolate

Target Value	Concentration [ppm]	Recovery [%]
10 ppm	6.20	100
5 ppm	3.27	106
2.5 ppm	1.64	106
1.25 ppm	0.72	92
0.63 ppm	0.34	86
	Mean [%]	**98**

Cornflakes

Target Value	Concentration [ppm]	Recovery [%]
10 ppm	8.94	100
5 ppm	4.07	91
2.5 ppm	2.45	110
1.25 ppm	1.05	94
0.63 ppm	0.40	71
	Mean [%]	**92**

Ice-cream

Target Value	Concentration [ppm]	Recovery [%]
10 ppm	7.97	100
5 ppm	3.72	93
2.5 ppm	1.74	87
1.25 ppm	0.75	75
0.63 ppm	0.29	58
	Mean [%]	**88**

For different matrices the mean linearity is ranging from 88% to 98%. The linearity is independent of the specific concentration and may only be affected by the intra-assay and inter-assay variation.

6. Cross-Reactivity

For the following foods no cross-reactivity (results < LOQ) could be detected:

Table 6: Non-cross-reactive food matrices in the AlerTox ELISA Almond

Milk	Corn	Cashew	Macadamia nut
Egg	Buckwheat	Peanut	Chestnut
Wheat	Soy	Hazelnut	Lecithin
Oats	Poppy seed	Pecan nut	Peach
Rye	Sesame	Brazil nut	Apricot
Barley	Sunflower seed	Coconut	Cherry
Cocoa	Pumpkin seed	Walnut	Plum
Rice	Pine seed	Pistachio	Almond

7. Robustness

Robustness was determined by variation of different handling parameters as defined in the instruction manual. The results were compared with the results of samples analyzed according to the intended method. An un-spiked cookie sample and a sample spiked with 2 ppm / 3 ppm of almond were analyzed respectively.

A) Variation of extraction temperature

The extraction temperature, defined as 60 °C, was changed to 25 °C, 40 °C and 70 °C, respectively.

Table 7: Variation of extraction temperature in the AlerTox ELISA Almond

Sample	Result 60 °C	Result 25 °C	Result 40 °C	Result 70 °C
Cookies 0 ppm	0 ppm	0 ppm	0 ppm	0 ppm
Cookies 2 ppm	1.54 ppm	1.62 ppm	1.77 ppm	1.76 ppm

Under consideration of the intra-assay and inter-assay variations, the results do not differ significantly.

B) Variation of extraction time

The extraction time, defined as 15 min, was changed to 10 min and 20 min, respectively.

Table 8: Variation of extraction time in the AlerTox ELISA Almond

Sample	Result 15 min	Result 10 min	Result 20 min
Cookies 0 ppm	0 ppm	0 ppm	0 ppm
Cookies 2 ppm	1.54 ppm	1.69 ppm	1.68 ppm

Under consideration of the intra-assay and inter-assay variations, the results do not differ significantly.

C) Drift

In contrast to the test procedure as 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 9: Drift in the AlerTox ELISA Almond

Sample	Result 20 min	Result 16 min	Result 24min
Cookies 0 ppm	0 ppm	0 ppm	0 ppm
Cookies 3 ppm	3.10 ppm	2.72 ppm	3.52 ppm

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