

Validation Report

AlerTox ELISA Cashew

KIT3053/KT-5916

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1. Scope

The AlerTox ELISA Cashew is designed for the determination of cashew 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 based on measured ppm of the AlerTox ELISA Cashew

Replicate	Level 1 [ppm]	Level 2 [ppm]	Level 3 [ppm]	
1	7.12	21.4	53.2	
2	6.67	20.0	51.9	
3	6.65	19.2	51.2	
4	7.04	20.9	53.3	
5	6.90	20.6	52.2	
6	6.64	20.4	49.8	
7	6.87	20.5	52.8	
8	6.64	18.1	46.6	
9	7.14	21.2	51.7	
10	6.60	19.9	49.1	
11	6.59	20.2	48.8	
12	6.99	21.5	51.0	
13	7.19	20.4	50.6	
14	6.52	19.7	47.9	
15	7.17	20.4	51.9	
16	6.34	18.3	44.4	
17	7.19	20.7	51.9	
18	6.80	19.5	51.0	
19	6.72	19.2	50.9	
20	6.92	20.3	52.3	
Mean	6.83	20.1	50.6	
SD	0.25	0.92	2.28	RMS
CV [%]	3.7	4.6	4.5	4.3

The coefficient of variation is ranging from 3.7% to 4.6% depending on the concentration.

RMS = Root Mean Square

Table 2: Intra-assay variation based on measured OD values of the AlerTox ELISA Cashew

Replicate	Level 1 OD _{450nm}	Level 2 OD _{450nm}	Level 3 OD _{450nm}	
1	0.466	1.197	2.422	
2	0.439	1.133	2.387	
3	0.438	1.176	2.367	
4	0.461	1.161	2.424	
5	0.453	1.153	2.394	
6	0.437	1.158	2.327	
7	0.451	1.189	2.415	
8	0.437	1.131	2.380	
9	0.467	1.144	2.306	
10	0.435	1.201	2.299	
11	0.434	1.154	2.362	
12	0.458	1.122	2.349	
13	0.470	1.152	2.271	
14	0.430	1.164	2.386	
15	0.469	1.114	2.162	
16	0.419	1.148	2.386	
17	0.470	1.136	2.361	
18	0.447	1.109	2.358	
19	0.442	1.147	2.398	
20	0.454	1.099	2.358	
Mean	0.449	1.149	2.356	
SD	0.015	0.028	0.061	RMS
CV [%]	3.4	2.4	2.6	2.8

The coefficient of variation is ranging from 2.4% to 3.4% depending on the concentration.

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 3: Inter-assay variation based on measured ppm of the AlerTox ELISA Cashew

Assay No.	Level 1 [ppm]	Level 2 [ppm]	Level 3 [ppm]	
1	6.50	20.8	46.7	
2	6.49	20.5	52.37	
3	6.61	20.8	50.6	
4	6.23	19.6	53.4	
Mean	6.46	20.43	50.8	
SD	0.16	0.60	2.94	RMS
CV [%]	2.5	2.9	5.8	4.0

The coefficient of variation is ranging from 2.5% to 5.8% depending on the concentration.

Table 4: Inter-assay variation based on measured OD values of the AlerTox ELISA Cashew

Assay No.	Level 1 OD _{450nm}	Level 2 OD _{450nm}	Level 3 OD _{450nm}	
1	0.480	1.287	2.787	
2	0.493	1.311	2.654	
3	0.474	1.265	2.526	
4	0.483	1.266	2.727	
Mean	0.483	1.282	2.674	
SD	0.008	0.022	0.112	RMS
CV [%]	1.6	1.7	4.2	2.8

The coefficient of variation is ranging from 1.6% to 4.2% depending on the concentration.

3. Recovery

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

Table 5: Recovery of various samples tested with the AlerTox ELISA Cashew

Cookies

Target Value	Actual Concentration	Recovery [%]
6 ppm	7.30	122
20 ppm	21.7	108
50 ppm	48.4	97
	Mean	109

Cornflakes

Target Value	Actual Concentration	Recovery [%]
6 ppm	6.30	105
20 ppm	20.4	102
50 ppm	43.1	86
	Mean	98

Ice-cream

Target Value	Actual Concentration	Recovery [%]
6 ppm	6.33	106
20 ppm	17.8	89
50 ppm	42.4	85
	Mean	93

Dark Chocolate

Target Value	Actual Concentration	Recovery [%]
6 ppm	7.26	121
20 ppm	18.9	95
50 ppm	44.8	90
	Mean	102

Mean recoveries are ranging from 93% to 109% depending on the sample matrix.

4. Analytical Sensitivity

For determination of the analytical sensitivity sample diluent and cashew free cookies, cornflakes, ice-cream and dark chocolate samples respectively were assayed in 24fold replicates. After identification of possible outliers the OD mean

and standard deviation were 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 6: Matrix-dependent and matrix-independent analytical sensitivity of the AlerTox ELISA Cashew

Replicate	Sample diluent [OD]	Cookie matrix [OD]	Cornflakes matrix [OD]	Ice-cream matrix [OD]	Dark Chocolate matrix [OD]
1	0.019	0.023	0.039	0.038	0.030
2	0.018	0.030	0.034	0.037	0.055
3	0.016	0.032	0.029	0.039	0.052
4	0.021	0.034	0.040	0.042	0.045
5	0.019	0.031	0.037	0.038	0.048
6	0.017	0.030	0.034	0.038	0.040
7	0.020	0.032	0.037	0.036	0.041
8	0.017	0.031	0.031	0.035	0.034
9	0.021	0.037	0.038	0.039	0.063
10	0.028	0.032	0.036	0.039	0.062
11	0.015	0.029	0.028	0.035	0.051
12	0.020	0.032	0.039	0.040	0.057
13	0.019	0.034	0.038	0.039	0.049
14	0.018	0.031	0.035	0.036	0.040
15	0.017	0.034	0.038	0.035	0.038
16	0.018	0.032	0.030	0.035	0.037
17	0.022	0.034	0.036	0.039	0.066
18	0.022	0.027	0.034	0.041	0.060
19	0.021	0.031	0.030	0.038	0.054
20	0.020	0.033	0.039	0.039	0.057
21	0.021	0.036	0.040	0.038	0.051
22	0.019	0.033	0.031	0.040	0.041
23	0.022	0.033	0.033	0.045	0.041
24	0.018	0.033	0.037	0.040	0.038
Mean	0.020	0.032	0.035	0.038	0.048
SD	0.003	0.003	0.004	0.002	0.010
Limit of Detection	0.2 ppm	0.0 ppm	0.3 ppm	0.3 ppm	0.5 ppm

The limit of detection (LOD) is 0.2 ppm of cashew. With respect to the sample matrix limits of detection vary from 0.0 to 0.5 ppm. Note that the derived limits of detection are strictly dependent on the coefficient of variation and may thus vary in every individual test. The data for sample diluent and matrices respectively were not determined in the same test runs.

The lowest positive standard (2 ppm) was defined as limit of quantification (LOQ) to assure that all uncontaminated matrices result in concentrations lower than

this value.

5. Linearity

Linearity was determined by spiking cookie, cornflakes, ice-cream and dark chocolate samples with cashew 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 7: Matrix dependent linearity of the AlerTox ELISA Cashew

Cookies

Target Value	Concentration [ppm]	Recovery [%]
50 ppm	48.44	100
25 ppm	28.17	116
12.5 ppm	15.50	128
6.25 ppm	7.44	123
3.125 ppm	3.97	131
	Mean [%]	125

Cornflakes

Target Value	Concentration [ppm]	Recovery [%]
50 ppm	63.04	100
25 ppm	33.46	106
12.5 ppm	17.15	109
6.25 ppm	8.20	104
3.125 ppm	4.29	109
	Mean [%]	107

Ice-cream

Target Value	Concentration [ppm]	Recovery [%]
50 ppm	46.53	100
25 ppm	24.19	104
12.5 ppm	13.42	115
6.25 ppm	6.46	111
3.125 ppm	3.62	124
	Mean [%]	114

Dark Chocolate

Target Value	Concentration [ppm]	Recovery [%]
50 ppm	44.83	100
25 ppm	26.39	118
12.5 ppm	14.73	131
6.25 ppm	7.37	131
3.125 ppm	3.96	141
	Mean [%]	131

For different matrices the mean linearity is ranging from 107% to 131%. The linearity is independent of the specific concentration and may only be affected by the intra-assay and inter-assay variation.

6. Cross-Reactivity

The following cross-reactivities could be determined:

Table 8: Cross-reactive food matrices in the AlerTox ELISA Cashew

Raw material	Cross-reactivity
Pistachio	4%

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

Table 9: Non-cross-reactive food matrices in the AlerTox ELISA Cashew

Almond	Chickpea	Lupine	Rice
Apricot	Cocoa	Macadamia	Rye
Barley	Coconut	Mustard	Sucrose
Bean	Cod	Oats	Sesame
Beef	Corn	Pea	Sheep's milk
Bovine gelatin	Cow's milk	Peanut	Shrimps, cooked
Brazil nut	Egg	Pecan	Shrimps, raw
Buckwheat	Gliadin	Pine seed	Soy
Carob gum	Goat's milk	Plum	Soy lecithin
Carrot	Guar gum	Poppy seed	Sunflower seed
Celery	Hazelnut	Pork meat	Tomato
Chestnut	Kiwi	Potato	Walnut
Chicken	Lentil	Pumpkin seed	Wheat

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 20 ppm of cashew 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 10: Variation of extraction temperature in the AlerTox ELISA Cashew

Sample	Result 60 °C	Result 25 °C	Result 40 °C	Result 70 °C
Cookie 0 ppm	0.00 ppm	0.03 ppm	0.00 ppm	0.00 ppm
Cookie 20 ppm	20.6 ppm	19,6 ppm	20,6 ppm	18.2 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 11: Variation of extraction time in the AlerTox ELISA Cashew

Sample	Result 15 min	Result 10 min	Result 20 min
Cookies 0 ppm	0.00 ppm	0.03 ppm	0.03 ppm
Cookies 20 ppm	20.6 ppm	22.0 ppm	20.4 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 12: Drift in the AlerTox ELISA Cashew

Sample	Result 20 min	Result 16 min	Result 24min
Cookies 0 ppm	0.00 ppm	0.00 ppm	0.00 ppm
Cookies 20 ppm	20.6 ppm	21.6 ppm	15.0 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.

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