Validation Report

AlerTox ELISA Macadamia
KIT3055/KT-6287

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1. Scope

The AlerTox ELISA Macadamia is designed for the determination of Macadamia 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 Macadamia

Replicate	Level 1 [ppm]	Level 2 [ppm]	Level 3 [ppm]	
1	4.62	10.4	39.1	
2	4.26	8.8	39.8	
3	3.89	11.6	36.6	
4	4.62	8.9	33.8	
5	4.43	9.9	37.9	
6	4.03	9.2	39.9	
7	4.21	10.3	40.8	
8	3.92	9.8	38.0	
9	4.71	10.6	40.0	
10	4.45	10.5	37.7	
11	4.57	10.2	37.5	
12	4.58	10.0	41.3	
13	4.51	10.2	32.3	
14	4.31	10.0	40.5	
15	4.36	9.3	37.0	
16	3.71	9.2	37.7	
17	3.93	10.0	39.2	
18	4.35	9.2	38.9	
9	4.24	10.5	33.9	
20	4.02	9.8	36.6	
Mean	4.29	9.9	33.8	
SD	0.29	0.67	2.42	**RMS**
CV [%]	**6.7**	**6.8**	**7.2**	**6.9**

The coefficient of variation is ranging from 6.7% to 7.2% depending on the concentration.

RMS = Root Mean Square

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

Replicate	Level 1 OD$_{450nm}$	Level 2 OD$_{450nm}$	Level 3 OD$_{450nm}$	
1	0.549	0.979	1.980	
2	0.515	1.018	1.992	
3	0.479	0.999	1.932	
4	0.549	0.892	1.876	
5	0.531	1.073	1.957	
6	0.492	0.896	1.993	
7	0.510	0.963	2.009	
8	0.482	0.921	1.959	
9	0.560	0.989	1.996	
10	0.533	0.959	1.953	
11	0.544	1.009	1.950	
12	0.545	1.002	2.018	
13	0.538	0.984	1.841	
14	0.519	0.971	2.004	
15	0.524	0.984	1.940	
16	0.461	0.972	1.954	
17	0.483	0.927	1.982	
18	0.523	0.917	1.975	
19	0.513	1.093	1.878	
20	0.491	0.973	1.933	
Mean	0.517	0.859	1.956	
SD	0.028	0.051	0.047	**RMS**
CV [%]	**5.4**	**6.0**	**2.4**	**4.7**

The coefficient of variation is ranging from 2.4% to 6.0% 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 Macadamia

Assay No.	Level 1 [ppm]	Level 2 [ppm]	Level 3 [ppm]	
1	4.50	10.8	44.0	
2	4.31	11.1	37.8	
3	4.24	10.5	41.4	
4	4.20	10.4	39.8	
Mean	4.31	10.72	40.75	
SD	0.13	0.29	2.62	RMS
CV [%]	3.1	2.7	6.4	4.4

The coefficient of variation is ranging from 2.7% to 6.4% depending on the concentration.

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

Assay No.	Level 1 OD_{450nm}	Level 2 OD_{450nm}	Level 3 OD_{450nm}	
1	0,378	0,728	2,385	
2	0,372	0,738	2,126	
3	0,389	0,760	2,285	
4	0,455	0,863	2,246	
Mean	0,399	0,772	2,261	
SD	0,038	0,062	0,107	RMS
CV [%]	9,6	8,0	4,7	7.7

The coefficient of variation is ranging from 4.7% to 9.6% depending on the concentration.

3. Recovery

For recovery experiments different sample matrices were spiked with macadamia nut to obtain various final concentrations after performing all sample pretreatment steps. Tested samples and results were as follows.

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

Cookies

Target Value	Actual Concentration	Recovery [%]
4 ppm	4.46	112
10 ppm	11.25	113
40 ppm	38.17	95
	Mean	106

Cornflakes

Target Value	Actual Concentration	Recovery [%]
4 ppm	4.36	109
10 ppm	11.00	110
40 ppm	36.70	92
	Mean	104

Ice-cream

Target Value	Actual Concentration	Recovery [%]
4 ppm	3.60	90
10 ppm	9.40	94
40 ppm	37.15	93
	Mean	92

Chocolate

Target Value	Actual Concentration	Recovery [%]
4 ppm	3.65	91
10 ppm	8.29	83
40 ppm	30.52	76
	Mean	83

Sausage

Target Value	Actual Concentration	Recovery [%]
4 ppm	4.17	104
10 ppm	10.89	109
40 ppm	35.89	90
	Mean	101

Mean recoveries are ranging from 83% to 106% depending on the sample matrix.

4. Analytical Sensitivity

For determination of the analytical sensitivity sample diluent and macadamia-free cookies, cornflakes, ice-cream, chocolate and sausage 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 Macadamia

Replicate	Sample diluent [OD]	Cookie matrix [OD]	Cornflakes matrix [OD]	Ice-cream matrix [OD]	Chocolate matrix [OD]	Sausage matrix [OD]
1	0.064	0.073	0.067	0.075	0.088	0.064
2	0.056	0.071	0.063	0.079	0.082	0.056
3	0.055	0.075	0.054	0.074	0.080	0.055
4	0.055	0.079	0.058	0.078	0.085	0.055
5	0.047	0.071	0.053	0.084	0.078	0.047
6	0.045	0.065	0.055	0.077	0.076	0.045
7	0.059	0.072	0.060	0.079	0.083	0.059
8	0.044	0.068	0.053	0.070	0.078	0.044
9	0.056	0.072	0.064	0.070	0.081	0.056
10	0.051	0.076	0.062	0.075	0.086	0.051
11	0.049	0.077	0.056	0.073	0.079	0.049
12	0.051	0.078	0.060	0.077	0.086	0.051
13	0.046	0.073	0.054	0.066	0.087	0.046
14	0.045	0.074	0.056	0.069	0.079	0.045
15	0.047	0.074	0.061	0.084	0.091	0.047
16	0.043	0.063	0.058	0.070	0.076	0.043

17	0.058	0.076	0.065	0.069	0.090	0.070
18	0.057	0.071	0.051	0.064	0.126	0.058
19	0.053	0.076	0.066	0.064	0.098	0.057
20	0.048	0.077	0.051	0.064	0.083	0.053
21	0.044	0.067	0.060	0.062	0.082	0.048
22	0.053	0.064	0.056	0.060	0.082	0.044
23	0.052	0.060	0.059	0.067	0.075	0.053
24	0.057	0.059	0.057	0.063	0.064	0.052
Mean	**0.051**	**0.071**	**0.058**	**0.071**	**0.084**	**0.052**
SD	**0.006**	**0.006**	**0.005**	**0.007**	**0.011**	**0.007**
Limit of Detection	**0.09 ppm**	**0.20 ppm**	**0.08 ppm**	**0.22 ppm**	**0.40 ppm**	**0.09 ppm**

With respect to the sample matrix limits of detection vary from 0.09 to 0.40 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 (1 ppm) was defined as limit of quantification to assure that all uncontaminated matrices result in concentrations lower than this value.

5. Linearity

Linearity was determined by spiking cookies, cornflakes, ice-cream, chocolate and sausage samples with macadamia 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 percent of this reference after consideration of the dilution factor.

Table 7: Matrix dependent linearity of the AlerTox ELISA Macadamia

Cookies

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	43.10	100
20 ppm	21.31	99
10ppm	11.03	102
5 ppm	5.52	102
2.5 ppm	2.81	104
	Mean [%]	102

Cornflakes

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	42.70	100
20 ppm	22.18	104
10ppm	11.44	107
5 ppm	5.76	108
2.5 ppm	2.78	104
	Mean [%]	106

Ice-cream

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	32.27	100
20 ppm	17.49	108
10ppm	9.69	120
5 ppm	4.59	114
2.5 ppm	2.12	105
	Mean [%]	112

Chocolate

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	32.73	100
20 ppm	17.60	108
10ppm	8.51	104
5 ppm	4.03	98
2.5 ppm	2.26	110
	Mean [%]	105

Sausage

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	35.31	100
20 ppm	20.40	116
10ppm	9.33	106
5 ppm	4.93	112
2.5 ppm	2.37	108
	Mean [%]	110

For different matrices the mean linearity is ranging from 102% to 112%. The linearity is independent of the specific concentration and may only be affected by the intra-assay and inter-assay variation as stated in chapter 2.

6. Cross-Reactivity

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

Table 8: Non-cross-reactive food matrices in the AlerTox ELISA Macadamia

Raw material	c [ppm]	Raw material	c [ppm]
Almond	0.08	Goat's milk	0.00
Apricot	0.01	Guar gum	0.00
Barley	0.19	Isinglass	0.01
Bean. white	0.01	Kiwi	0.00
Beef	0.25	Lamb	0.01
Bovine gelatin	0.00	Lentil	0.08
Brazil nut	0.14	Lupine	0.08
Buckwheat	0.18	Mustard	0.22
Carob gum	0.00	Oats	0.18
Carrot	0.44	Pea	0.00
Cashew	0.08	Peach	0.18
Celery	0.29	Peanut	0.49
Cherry	0.19	Pecan	0.86
Chervil	0.26	Pine seed	0.01
Chestnut	0.20	Pistachio	0.26
Chicken	0.17	Plum	0.00
Chickpea	0.06	Poppy seed	0.29
Cocoa	0.15	Pork	0.00
Coconut	0.51	Potato	0.00
Cod	0.10	Pumpkin seed	0.44
Corn	0.04	Rice	0.12
Cow' milk	0.00	Rye	0.12
Crab. cooked	0.00	Sucrose	0.02
Crab. rob	0.00	Sesame	0.31
Cress	0.00	Soy	0.09
Egg	0.04	Soy lecithin	0.08
Egg white powder	0.04	Sunflower seeds	0.41
Ewe's milk	0.06	Tofu	0.00
Fish gelatin	0.00	Tomato	0.00
Gliadin	0.39	Wheat	0.23

The following cross-reactivities could be determined:
Table 9: Cross-reactive food matrices in the AlerTox ELISA Macadamia

Food matrix	Cross-reactivity [%]
Hazelnut	0.0002
Walnut	0.0010

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 unspiked cookie sample and a sample spiked with 10 ppm of macadamia nut 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 Macadamia

Sample	Result 60 °C	Result 25 °C	Result 40 °C	Result 70 °C
Cookie 0 ppm	0.1 ppm	0.1 ppm	0.0 ppm	0.0 ppm
Cookie 10 ppm	10.5 ppm	11.0 ppm	10.5 ppm	11.2 ppm

Under consideration of the intra-assay and inter-assay variations, the results do <u>not</u> 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 Macadamia

Sample	Result 15 min	Result 10 min	Result 20 min
Cookie 0 ppm	0.0 ppm	0.0 ppm	0.0 ppm
Cookie 10 ppm	8.1 ppm	11.7 ppm	9.8 ppm

Under consideration of the intra-assay and inter-assay variations, the results do <u>not</u> 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 Macadamia

Sample	Result 20 min	Result 16 min	Result 24min
Cookie 0 ppm	0.0 ppm	0.0 ppm	0.0 ppm
Cookie 10 ppm	10.3 ppm	12.8 ppm	10.0 ppm

The results do not 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.