

Validation Report

AlerTox ELISA Hazelnut KIT3050/KT-5907

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1. Scope

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

Replicate	Level 1	Level 2	Level 3	
1	2.3	10.7	33.4	
2	2.3	10.0	33.2	
3	2.2	10.1	34.5	
4	2.8	10.1	33.1	
5	2.9	10.4	35.5	
6	2.2	8.6	30.5	
7	2.9	8.3	32.4	
8	2.7	8.1	29.0	
9	2.2	9.6	31.6	
10	2.7	10.6	28.9	
11	2.4	10.2	28.7	
12	2.9	10.5	29.3	
13	2.9	11.0	28.7	
14	2.3	10.9	28.9	
15	3.0	9.0	28.9	
16	2.8	7.8	27.8	
17	2.2	11.3	31.4	
18	2.3	9.7	28.1	
19	2.2	8.1	27.7	
20	2.9	10.0	29.1	
Mean	2.5	9.7	30.5	
SD	0.30	1.07	2.41	Mean
CV [%]	11.9	10.9	7.9	10.3

The coefficient of variation is ranging from 7.9% to 11.9% 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 2: Inter-assay variation of the AlerTox ELISA Hazelnut

Assay No.	Level 1	Level 2	Level 3	
1	2.4	8.0	30.5	
2	2.3	7.3	32.2	
3	2.3	9.5	35.9	
4	2.5	9.6	37.1	
Mean	2.4	8.6	33.9	
SD	0.08	1.13	3.08	Mean
VK [%]	3.2	13.2	9.1	8.5

The coefficient of variation is ranging from 3.2% to 13.2% depending on the concentration.

3. Recovery

For recovery experiments different sample matrices were spiked with hazelnut 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 Hazelnut

Cookies

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.8	95
15 ppm	15.9	106
	Mean	101

Cornflakes

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.5	91
15 ppm	16.2	108
	Mean	99

Chocolate

Target Value	Actual Concentration	Recovery [%]
5 ppm	3.9	78
15 ppm	13.0	87
	Mean	83

Dark Chocolate

Target Value	Actual Concentration	Recovery [%]
5 ppm	3.8	76
15 ppm	11.7	78
	Mean	77

Ice-cream

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.3	86
15 ppm	13.9	93
	Mean	90

Brazil nut

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.7	94
15 ppm	14.8	99
	Mean	97

Mean recoveries are ranging from 77% to 101% 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 Hazelnut

Replicate	Sample diluent [OD]
1	0.083
2	0.081
3	0.093
4	0.087
5	0.080
6	0.081
7	0.095
8	0.114
9	0.077
10	0.078
11	0.096
12	0.086
13	0.075
14	0.078
15	0.081
16	0.087
17	0.075
18	0.078
19	0.080
20	0.087
21	0.083
22	0.075
23	0.087
24	0.087
Mean	0.084
SD	0.009
Limit of Detection	0.3 ppm

The limit of detection is 0.3 ppm of hazelnut.

The lowest positive standard (1 ppm) was defined as limit of quantification to assure that all important matrices like milk, wheat, rye, oats, barley, egg. and cocoa result in concentrations lower than this value.

5. Linearity

Linearity was determined by spiking cookies, chocolate, cornflakes and ice-cream samples with hazelnut 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 5: Matrix dependent linearity of the AlerTox ELISA Hazelnut

Cookies

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	40.4	100
20 ppm	17.5	87
10 ppm	9.7	96
5 ppm	5.5	109
2.5 ppm	2.8	111
	Mean [%]	101

Chocolate

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	33.8	100
20 ppm	17.3	102
10 ppm	8.5	100
5 ppm	4.7	110
2.5 ppm	2.6	121
	Mean [%]	108

Cornflakes

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	40.1	100
20 ppm	19.2	96
10 ppm	9.3	93
5 ppm	4.9	98
2.5 ppm	2.6	105
	Mean [%]	98

Ice-cream

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	35.7	100
20 ppm	18.3	102
10 ppm	9.2	103
5 ppm	5.1	113
2.5 ppm	2.6	118
	Mean [%]	109

For different matrices the mean linearity is ranging from 98% to 109%. 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 6: Non-cross-reactive food matrices in the AlerTox ELISA Hazelnut

Wheat	Chickpea	Sesame	Cocoa
Barley	Bean	Peanut	Milk
Rye	Soy	Chestnut	Gluten
Oats	Poppy seed	Coconut	Soy lecithin
Buckwheat	Sunflower seed	Brazil nut	Gelatin
Corn	Pumpkin seed	Pecan nut	Apple
Rice	Pine seed	Pistachio	
Egg	Cashew	Macadamia nut	

The following cross-reactivity could be determined: Walnut < 0.0022%

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 hazelnut were analyzed respectively.

Variation of extraction temperature

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

Table 8: Variation of extraction temperature in the AlerTox ELISA Hazelnut

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 10 ppm	9.7 ppm	9.5 ppm	9.7 ppm	10.5 ppm

Under consideration of the intra-assay and inter-assay variations as stated in chapter 2 the results do not differ significantly.

Variation of extraction time

The extraction time, defined as 15 min, was changed to 5 min. 10 min and 20 min. respectively.

Table 9: Variation of extraction time in the AlerTox ELISA Hazelnut

Sample	Result 15 min	Result 5 min	Result 10 min	Result 20 min
Cookies 0 ppm	0 ppm	0 ppm	0 ppm	0 ppm
Cookies 10 ppm	9.7 ppm	9.2 ppm	9.8 ppm	9.1 ppm

Under consideration of the intra-assay and inter-assay variation as stated in chapter 2, the results do not differ significantly.

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 10: Drift in the AlerTox ELISA Hazelnut

Sample	Result 20 min	Result 16 min	Result 24min
Cookies 0 ppm	0 ppm	0 ppm	0 ppm
Cookies 10 ppm	9.0 ppm	8.1 ppm	9.2 ppm

Under consideration of the intra-assay and inter-assay variations as stated in chapter 2, the results do not differ significantly. Anyway, 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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