

Validation Report

AlerTox ELISA Walnut KIT3052/KIT-5909

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1. Scope

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

Replicate	Level 1	Level 2	
1	4.8	15.7	
2	4.7	17.5	
3	4.9	16.5	
4	4.7	15.4	
5	4.8	16.9	
6	4.2	15.4	
7	4.6	16.5	
8	3.9	15.9	
9	6.1	17.1	
10	4.9	17.2	
11	4.8	17.1	
12	4.7	16.4	
13	4.7	16.6	
14	4.7	15.9	
15	4.7	16.4	
16	4.6	15.9	
17	4.9	17.9	
18	4.6	17.0	
19	4.6	16.0	
20	4.3	16.8	
Mean	4.4	16.5	
SD	0.41	0.69	Mean
CV [%]	9.3	4.2	6.8

The coefficient of variation is ranging from 4.2% to 9.3% 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 Walnut

Assay No.	Level 1	Level 2	
1	5.7	18.8	
2	4.7	18.5	
3	6.3	14.5	
4	5.8	15.9	
Mean	5.6	16.9	
SD	0.65	2.08	Mean
VK [%]	11.5	12.3	11.9

The coefficient of variation is ranging from 11.5% to 12.3% depending on the concentration.

3. Recovery

For recovery experiments, different sample matrices were spiked with Walnut 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 Walnut

Cornflakes

Target Value	Actual Concentration	Recovery [%]
6 ppm	6.8	113
20 ppm	18.3	92
60 ppm	67.7	113
	Mean	106

Cookies

Target Value	Actual Concentration	Recovery [%]
6 ppm	5.6	94
20 ppm	17.5	88
60 ppm	77.2	129
	Mean	103

Ice-cream

Target Value	Actual Concentration	Recovery [%]
6 ppm	5.2	86
20 ppm	14.9	75
60 ppm	61.0	102
	Mean	87

Chocolate

Target Value	Actual Concentration	Recovery [%]
6 ppm	4.5	75
20 ppm	9.3	46
60 ppm	35.5	59
	Mean	60

Mean recoveries are ranging from 60% to 106% 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 limit of detection according to the following table:

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

Replicate	Sample diluent [OD]
1	0.222
2	0.217
3	0.214
4	0.253
5	0.222
6	0.208
7	0.213
8	0.193
9	0.220
10	0.229
11	0.214
12	0.206
13	0.227
14	0.187
15	0.205
16	0.192
17	0.242
18	0.144
19	0.210
20	0.201
21	0.221
22	0.217
23	0.215
24	0.200
Mean	0.211
SD	0.021
Limit of Detection	0.4 ppm

The limit of detection is 0.4 ppm of walnut.

The lowest positive standard (2 ppm) was defined as limit of quantification to assure that all important matrices like milk, wheat, rye, oats, barley, egg, beef, chicken and pork meat result in concentrations lower than this value.

5. Linearity

Linearity was determined by spiking ice-cream, cookie, cornflakes and chocolate samples with walnut 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 Walnut

Ice-cream

Target Value	Concentration [ppm]	Recovery [%]
60 ppm	61.0	100
30 ppm	38.5	126
15 ppm	20.4	134
7.5 ppm	6.1	80
3.75 ppm	3.2	84
	Mean [%]	106

Cookies

Target Value	Concentration [ppm]	Recovery [%]
60 ppm	77.2	100
30 ppm	49.3	128
15 ppm	18.1	94
7.5 ppm	7.6	79
3.75 ppm	3.4	70
	Mean [%]	93

Cornflakes

Target Value	Concentration [ppm]	Recovery [%]
60 ppm	67.7	100
30 ppm	49.3	146
15 ppm	18.6	110
7.5 ppm	8.8	104
3.75 ppm	3.6	85
	Mean [%]	111

Chocolate

Target Value	Concentration [ppm]	Recovery [%]
60 ppm	39.1	100
30 ppm	18.5	95
15 ppm	11.1	114
7.5 ppm	6.4	131
3.75 ppm	3.1	128
	Mean [%]	117

For different matrices the mean linearity is ranging from 93% to 117%. 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 Walnut

Adzuki	Chickpea	Guar gum	Pistachio
Almond	Chili	Hazelnut	Plum
Apricot	Cocoa	Isinglass	Poppy seed
Barley	Coconut	Kidney bean	Pork
Bean, white	Cod	Kiwi	Potato
Beef	Corn	Lamb	Pumpkin seed
Bovine gelatin	Cow's milk	Lentil	Rice
Brazil nut	Crab, cooked	Lupin	Rye
Buckwheat	Crab, raw	Macadamia	Saccharose
Caraway	Cress	Mustard	Sesame
Carob gum	Cumin	Nutmeg	Shrimp, cooked
Carrot	Duck	Oats	Shrimp, raw
Cayenne	Egg	Onion	Soy flour
Celery	Ewe's milk	Paprika	Sunflower seeds
Cherry	Fenugreek	Pea	Tomato
Chervil	Fish gelatin	Peanut	Turkey
Chestnut	Gliadin	Pepper	Wheat
Chia	Goat's milk	Pine seed	

The following cross-reactivities could be determined:

Table 7: Cross-reactive food matrices in the AlerTox ELISA Walnut

Food	Cross-reactivity [%]
Cashew	0.0002%
Chicken	0.0002%
Pecan	0.0005%
Cashew	0.0002%

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 cookies sample and a sample spiked with 10 ppm of walnut were analyzed respectively.

Variation of extraction temperature

The extraction temperature, defined as 60 °C, was changed to 20 °C, 40 °C and 70 °C, respectively.

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

Sample	Result 60 °C	Result 20 °C	Result 40 °C	Result 70 °C
Cookies 0 ppm	0 ppm	0.2 ppm	0 ppm	0 ppm
Cookies 10 ppm	9,8	11.9	7.9	5.7 ppm

The results show that extraction temperature should not exceed 60 °C. Under consideration of the intra-assay and inter-assay variation as stated in chapter 2, the results of temperature < 60 °C do not differ significantly.

Variation of extraction time

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

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

Sample	Result 15 min	Result 10 min	Result 20 min
Cookies 0 ppm	0 ppm	0 ppm	0 ppm
Cookies 10 ppm	9.8 ppm	11.2 ppm	9.8 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 Walnut

Sample	Result 20 min	Result 16 min	Result 24min
Cookies 0 ppm	0 ppm	0 ppm	0 ppm
Cookies 10 ppm	9.8 ppm	6.7 ppm	14.6 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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