

Validation Report

AlerTox ELISA Peanut

KIT3048/KT-5905

INDEX

1. Scope	2
2. Precision	2
A) Intra-Assay Variation	2
B) Inter-Assay Variation	3
3. Recovery	3
4. Analytical Sensitivity	4
5. Linearity	5
6. Cross-Reactivity	7
7. Robustness	7

1. Scope

The AlerTox ELISA Peanut is designed for the determination of peanut 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 Peanut

Replicate	Level 1	Level 2	Level 3	
1	2.3	12.5	41.5	
2	2.4	12.3	46.3	
3	2.7	11.9	43.7	
4	2.7	13.0	44.4	
5	2.9	12.8	49.3	
6	2.7	12.6	42.1	
7	3.0	12.3	43.6	
8	3.0	12.7	39.4	
9	2.6	11.8	41.9	
10	2.6	12.3	42.0	
11	3.0	11.8	43.0	
12	3.1	12.7	46.2	
13	3.1	10.6	45.5	
14	2.7	10.7	38.6	
15	3.2	11.0	38.6	
16	3.1	10.0	37.6	
17	2.5	11.8	37.2	
18	3.2	12.2	38.8	
19	3.0	12.0	38.7	
20	3.2	12.9	39.3	
Mean	2.9	12.0	41.9	
SD	0.29	0.84	3.37	RMS
CV [%]	10.1	7.0	8.0	8.5

The coefficient of variation is ranging from 7.0% to 10.1% 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 Peanut

Assay No.	Level 1	Level 2	Level 3	
1	2.4	12.3	46.7	
2	2.3	10.6	52.8	
3	2.4	10.0	44.0	
4	2.3	10.6	40.9	
Mean	2.4	10.9	46.1	
SD	0.05	1.03	5.05	RMS
CV [%]	2.2	9.5	11.0	8.5

The coefficient of variation is ranging from 2.2% to 11.0% depending on the concentration.

3. Recovery

For recovery experiments different sample matrices were spiked with peanut 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 Peanut

Cornflakes

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.6	92
15 ppm	16.2	108
	Mean	100

Cookies

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.3	85
15 ppm	17.4	116
	Mean	101

Chocolate

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.7	94
15 ppm	18.9	126
	Mean	110

Ice-cream

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.2	84
15 ppm	14.3	95
	Mean	89

Hazelnut

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.9	98
15 ppm	14.2	95
	Mean	96

Mean recoveries are ranging from 89% to 110% 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 Peanut

Replicate	Sample diluent [OD]
1	0.053
2	0.051
3	0.050
4	0.054
5	0.049
6	0.052

Replicate	Sample diluent [OD]
7	0.056
8	0.054
9	0.050
10	0.051
11	0.053
12	0.054
13	0.049
14	0.060
15	0.047
16	0.047
17	0.047
18	0.049
19	0.054
20	0.051
21	0.048
22	0.053
23	0.049
24	0.049
Mean	0.051
SD	0.003
Limit of Detection	0.1 ppm

The limit of detection is 0.1 ppm of peanut. For safety reasons a cut-off was set to **0.3 ppm** for LOD. The lowest positive standard (1 ppm) was defined as limit of quantification (LOQ).

5. Linearity

Linearity was determined by spiking cookies, chocolate, cornflakes and ice-cream samples with peanut 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 Peanut

Cookies

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	39.25	100
20 ppm	18.89	96
10 ppm	10.39	106
5 ppm	4.61	94
2.5 ppm	2.39	97
	Mean [%]	98

Chocolate

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	36.55	100
20 ppm	15.72	86
10 ppm	9.39	103
5 ppm	3.89	85
2.5 ppm	2.44	107
	Mean [%]	95

Cornflakes

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	33.22	100
20 ppm	14.18	85
10 ppm	7.46	90
5 ppm	3.58	86
2.5 ppm	1.97	95
	Mean [%]	89

Ice-cream

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	32.84	100
20 ppm	18.12	110
10 ppm	7.62	93
5 ppm	4.10	100
2.5 ppm	2.36	115
	Mean [%]	105

For different matrices the mean linearity is ranging from 89% to 105%. 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 Peanut

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

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 10 ppm of peanut were analyzed respectively.

A) Variation of extraction temperature

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

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

Sample	Result 60 °C	Result 40 °C	Result 70 °C
Cookies 0 ppm	0 ppm	0 ppm	0 ppm
Cookies 10 ppm	11.3 ppm	12.5 ppm	9.1 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 5 min, 10 min and 20 min, respectively.

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

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	11.3 ppm	14.5 ppm	13.0 ppm	11.1 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 Peanut

Sample	Result 20 min	Result 16 min	Result 24min
Cookies 0 ppm	0 ppm	0 ppm	0 ppm
Cookies 10 ppm	9.7 ppm	7.9 ppm	10.8 ppm

Under consideration of the intra-assay and inter-assay variations, 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.

Date: Dec 2014