

Validation Report

AlerTox ELISA Egg

KIT3046/KIT-5904

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1. Scope

The AlerTox ELISA Egg is designed for the determination of egg white 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 Egg

Replicate	Level 1	Level 2	Level 3	
1	0.93	3.84	9.05	
2	0.97	4.09	8.62	
3	0.98	3.81	8.45	
4	0.90	3.78	8.17	
5	0.97	3.95	8.67	
6	1.06	3.81	8.92	
7	0.98	3.56	8.18	
8	0.91	3.45	8.90	
9	0.96	3.90	8.90	
10	0.96	3.90	outlier	
11	0.98	3.69	11.6	
12	0.96	3.77	8.98	
13	0.94	3.89	9.15	
14	0.95	3.99	8.91	
15	0.97	3.93	8.54	
16	0.95	3.87	8.72	
17	1.02	3.76	8.68	
18	0.98	3.83	9.34	
19	1.02	3.92	8.60	
20	1.06	3.76	7.80	
Mean	0.97	3.82	8.85	
SD	0.04	0.14	0.76	RMS
CV [%]	4.4	3.8	8.6	6.0

The coefficient of variation is ranging from 3.8% to 8.6% 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 Egg

Assay No.	Level 1	Level 2	Level 3	
1	0.88	3.68	9.08	
2	0.86	3.62	8.85	
3	0.93	4.17	9.43	
4	0.99	3.79	9.08	
Mean	0.91	3.82	9.11	
SD	0.06	0.25	0.24	RMS
CV [%]	6.5	6.4	2.6	5.5

The coefficient of variation is ranging from 2.6% to 6.5% depending on the concentration.

3. Recovery

For recovery experiments different sample matrices were spiked with egg white 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 Egg

Pasta

Target Value	Actual Concentration	Recovery [%]
1 ppm	0.87	87
4 ppm	3.78	95
10 ppm	9.20	92
	Mean	91

Biscuit

Target Value	Actual Concentration	Recovery [%]
1 ppm	0.85	85
4 ppm	3.71	93
10 ppm	7.14	71
	Mean	83

Cookies

Target Value	Actual Concentration	Recovery [%]
1 ppm	0.83	83
4 ppm	3.58	90
10 ppm	8.23	82
	Mean	85

Sausage

Target Value	Actual Concentration	Recovery [%]
1 ppm	0.95	95
4 ppm	4.02	101
10 ppm	9.87	99
	Mean	98

Dark Chocolate

Target Value	Actual Concentration	Recovery [%]
1 ppm	0.78	78
4 ppm	3.64	91
10 ppm	7.72	77
	Mean	82

Mean recoveries are ranging from 82% to 98% 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 $OD_{\text{mean}} + 3x$ 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 Egg

Replicate	Sample diluent [OD]
1	0.065
2	0.052
3	outlier
4	0.056
5	0.049
6	0.049
7	0.053
8	0.042
9	outlier
10	outlier
11	0.047
12	0.048
13	0.046
14	0.044
15	0.044
16	0.044
17	outlier
18	outlier
19	0.055
20	0.055
21	0.047
22	0.045
23	0.043
24	0.044
Mean	0.051
SD	0.013
Limit of Detection	0.05 ppm

The calculated limit of detection is 0.05 ppm of egg white.

The lowest positive standard (0.4 ppm) was defined as limit of quantification to assure that all important matrices like milk, wheat, rye, oats, barley, pork, beef, and cocoa result in concentrations lower than this value.

5. Linearity

Linearity was determined by spiking pasta, biscuit, cookies, sausage and chocolate, samples with egg white 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 Egg

Pasta

Target Value	Concentration [ppm]	Recovery [%]
10 ppm	9.73	100
5 ppm	5.30	109
2.5 ppm	2.76	113
1.25 ppm	1.25	103
0.63 ppm	0.57	94
	Mean [%]	105

Biscuit

Target Value	Concentration [ppm]	Recovery [%]
10 ppm	9.89	100
5 ppm	5.31	107
2.5 ppm	2.92	118
1.25 ppm	1.50	121
0.63 ppm	0.62	100
	Mean [%]	112

Cookies

Target Value	Concentration [ppm]	Recovery [%]
10 ppm	9.63	100
5 ppm	4.65	97
2.5 ppm	2.66	110
1.25 ppm	1.32	110
0.63 ppm	0.75	125
	Mean [%]	110

Sausage

Target Value	Concentration [ppm]	Recovery [%]
10 ppm	10.35	100
5 ppm	5.18	100
2.5 ppm	2.79	108
1.25 ppm	1.14	88
0.63 ppm	0.40	62
	Mean [%]	90

Chocolate

Target Value	Concentration [ppm]	Recovery [%]
10 ppm	8.60	100
5 ppm	4.09	95
2.5 ppm	2.43	113
1.25 ppm	0.96	89
0.63 ppm	0.41	76
	Mean [%]	93

For different matrices the mean linearity is ranging from 90% to 112%. 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) were detected:

Table 6: Non-cross-reactive food matrices in the AlerTox ELISA Egg

Milk	Cherry	Soy	Macadamia nut
Wheat	Sucrose	Apricot	Pistachio
Rye	Corn	Peach	Walnut
Oats	Cashew	Peanut	Coconut
Barley	Pine seed	Hazelnut	Brazil nut
Pork	Pumpkin seed	Buckwheat	Pecan nut
Beef	Sunflower seed	Rice	Plum
Cacao	Sesame	Lecithin	
Ewe`s milk	Poppy seed	Chestnut	

The following cross-reactivities could be determined:

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

Food	Cross-reactivity [%]
Chicken meat	0.001

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 4 ppm of egg white 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 8: Variation of extraction temperature in the AlerTox ELISA Egg

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 4 ppm	4.41 ppm	2.58 ppm	2.60 ppm	3.99 ppm

The results differ significantly. The extraction temperature should comply exactly with the temperature defined in the instruction manual as 60 °C.

B) 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 Egg

Sample	Result 15 min	Result 10 min	Result 20 min
Cookies 0 ppm	0 ppm	0 ppm	0 ppm
Cookies 4 ppm	4.41 ppm	4.44 ppm	3.66 ppm

Under consideration of the intra-assay and inter-assay variation, 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 10: Drift in the AlerTox ELISA Egg

Sample	Result 20 min	Result 16 min	Result 24min
Cookies 0 ppm	0 ppm	0 ppm	0 ppm
Cookies 4 ppm	4.41 ppm	2.70 ppm	4.32 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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