

Validation Report

AlerTox ELISA Lupine

KIT3057/KT-5914

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	7
6. Cross-Reactivity	8
7. Robustness	9

1. Scope

The AlerTox ELISA Lupine is designed for the determination of lupine 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 Lupine

Replicate	Level 1	Level 2	Level 3	
1	5.7	20	34	
2	6.1	18	36	
3	6.2	22	38	
4	6.2	19	37	
5	6.3	19	34	
6	6.3	20	35	
7	7.0	20	36	
8	5.7	16	31	
9	5.4	20	35	
10	5.7	20	39	
11	6.0	21	34	
12	5.6	21	35	
13	6.0	17	34	
14	6.2	19	32	
15	6.2	20	28	
16	6.1	18	29	
17	5.3	18	29	
18	5.8	20	30	
19	6.0	22	33	
20	6.3	21	31	
Mean	6.0	20	33	
SD	0.4	1.5	3.0	RMS
CV [%]	6.1	7.8	9.1	7.8

The coefficient of variation is ranging from 6.1% to 9.1% 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 Lupine

Assay No.	Level 1	Level 2	Level 3	
1	4.6	13	27	
2	5.1	13	22	
3	4.6	14	23	
4	4.6	14	25	
Mean	4.7	13	24	
SD	0.2	0.5	2.2	RMS
CV [%]	5.1	4.1	9.3	6.6

The coefficient of variation is ranging from 4.1% to 9.3% depending on the concentration.

3. Recovery

For recovery experiments different sample matrices were spiked with lupine 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 Lupine

Biscuit

Target Value	Actual Concentration	Recovery [%]
5 ppm	5.9	119
15 ppm	16.7	111
30 ppm	32.7	109
	Mean	113

Orange Juice

Target Value	Actual Concentration	Recovery [%]
5 ppm	6.1	122
15 ppm	15.1	100
30 ppm	26.4	88
	Mean	104

Sausage

Target Value	Actual Concentration	Recovery [%]
5 ppm	5.3	105
15 ppm	14.7	98
30 ppm	28.5	95
	Mean	99

Ketchup

Target Value	Actual Concentration	Recovery [%]
5 ppm	5.4	109
15 ppm	14.0	93
30 ppm	27.4	91
	Mean	98

Croquette

Target Value	Actual Concentration	Recovery [%]
5 ppm	6.4	127
15 ppm	16.3	109
30 ppm	28.9	96
	Mean	111

Mean recoveries are ranging from 98% to 113% depending on the sample matrix.

4. Analytical Sensitivity

a) Matrix-independent Sensitivity

For determination of the analytical sensitivity, matrix-independent 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 limit of detection according to the following table:

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

Replicate	Sample diluent [OD]
1	0.070
2	0.063
3	0.056
4	0.054
5	0.086
6	0.066
7	0.081
8	0.071
9	0.074
10	0.079
11	0.060
12	0.069
13	0.074
14	0.068
15	0.072
16	0.071
17	0.084
18	0.068
19	0.072
20	0.075
21	0.070
22	0.072
23	0.068
24	0.070
Mean	0.071
SD	0.008
Limit of Detection	0.21 ppm

b) Matrix-dependent Sensitivity

For determination of the analytical sensitivity, matrix-dependent lupine free biscuit orange juice, sausage, ketchup and croquette samples were assayed in 24fold replicates. After identification of possible outliers the OD mean and standard deviation were 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 4b: Matrix-dependent analytical sensitivity of the AlerTox ELISA Lupine

Replicate	Biscuit matrix [OD]	Orange Juice matrix [OD]	Sausage matrix [OD]	Ketchup matrix [OD]	Croquette matrix [OD]
1	0.121	0.356	0.089	0.094	0.099
2	0.125	0.321	0.090	0.066	0.091
3	0.114	0.336	0.083	0.081	0.102
4	0.120	0.342	0.078	0.070	0.098
5	0.106	0.330	0.068	0.077	0.081
6	0.105	0.330	0.073	0.080	0.083
7	0.112	0.341	0.073	0.098	0.106
8	0.098	0.291	0.063	0.079	0.087
9	0.106	0.334	0.085	0.083	0.113
10	0.102	0.303	0.084	0.070	0.102
11	0.113	0.320	0.076	0.082	0.096
12	0.113	0.367	0.079	0.074	0.105
13	0.127	0.333	0.084	0.085	0.084
14	0.105	0.321	0.067	0.078	0.091
15	0.096	0.332	0.077	0.074	0.093
16	0.116	0.298	0.066	0.062	0.080
17	0.095	0.344	0.093	0.080	0.104
18	0.131	0.317	0.083	0.079	0.104
19	0.115	0.320	0.074	0.074	0.091
20	0.114	0.305	0.074	0.072	0.089
21	0.125	0.301	0.073	0.068	0.082
22	0.115	0.321	0.069	0.073	0.104
23	0.113	0.325	0.081	0.078	0.092
24	0.125	0.309	0.062	0.071	0.093
Mean	0.113	0.325	0.077	0.077	0.095
SD	0.010	0.018	0.009	0.008	0.009
Limit of Detection	0.25 ppm	0.69 ppm	0.16 ppm	0.06 ppm	0.23 ppm
LOD_{mean}	0.278 ppm				

The calculated limit of detection is ranging from 0.21ppm to 0.278 ppm of lupine. 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 LOD was therefore set to 0.3 ppm.

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

5. Linearity

Linearity was determined by spiking biscuit, orange juice, sausage, ketchup and croquette samples with lupine 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 Lupine

Biscuit

Target Value	Concentration [ppm]	Recovery [%]
30 ppm	32.7	100
15 ppm	14.7	90
7.5 ppm	8.6	105
3.8 ppm	4.0	98
1.9 ppm	1.6	78
	Mean [%]	93

Orange Juice

Target Value	Concentration [ppm]	Recovery [%]
30 ppm	27.1	100
15 ppm	17.1	126
7.5 ppm	8.3	123
3.8 ppm	3.7	108
1.9 ppm	1.2	70
	Mean [%]	107

Sausage

Target Value	Concentration [ppm]	Recovery [%]
30 ppm	28.4	100
15 ppm	14.6	103
7.5 ppm	7.3	103
3.8 ppm	3.8	107
1.9 ppm	1.6	91
	Mean [%]	101

Ketchup

Target Value	Concentration [ppm]	Recovery [%]
30 ppm	27.4	100
15 ppm	14.3	104
7.5 ppm	7.8	114
3.8 ppm	4.0	117
1.9 ppm	1.5	90
	Mean [%]	106

Croquette

Target Value	Concentration [ppm]	Recovery [%]
30 ppm	28.9	100
15 ppm	16.1	112
7.5 ppm	7.5	103
3.8 ppm	3.8	106
1.9 ppm	1.7	95
	Mean [%]	104

For different matrices the mean linearity is ranging from 93% to 107%. 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 Lupine

Milk	Tomato	Hazelnut	Macadamia nut
Egg	Pork	Almond	Chestnut
Wheat	Sunflower seed	Cacao	Peach
Rye	Pumpkin seed	Potato	Cherry
Oats	Pine seed	Leech	Plum
Barley	Cashew	Pecan	Pea
Rice	Peanut	Brazil nut	Bean
Corn	Guar gum	Coconut	
Buckwheat	Locust bean gum	Walnut	
Sesame	Chicken	Pistachio	

The following cross-reactivities could be determined:

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

Food	Cross-reactivity [%]
Chickpea	0.0003
Soy, unroasted	0.07
Soy, roasted	0.0009
Soy lecithin*	0.002
Lentil	0.0004
Beef*	0.0003

*) Validated in an additionally validation run.

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 biscuit sample and a sample spiked with 5 ppm of lupine 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 8: Variation of extraction temperature in the AlerTox ELISA Lupine

Sample	Result 60 °C	Result 40 °C	Result 70 °C
Biscuit 0 ppm	0.15 ppm	0.13 ppm	0.22 ppm
Biscuit 5 ppm	4.47 ppm	4.73 ppm	4.18 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 10 min and 20 min. respectively.

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

Sample	Result 15 min	Result 10 min	Result 20 min
Biscuit 0 ppm	0.15 ppm	0.26 ppm	0.15 ppm
Biscuit 5 ppm	4.47 ppm	5.13 ppm	5.41 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 5 minutes compared to the calibrators (20 min).

Table 10: Drift in the AlerTox ELISA Lupine

Sample	Result 20 min	Result 15 min	Result 25 min
Biscuit 0 ppm	0.15 ppm	0.02 ppm	0.05 ppm
Biscuit 5 ppm	4.47 ppm	2.85 ppm	6.55 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.