

Validation Report

AlerTox ELISA Soy

KIT3047/KT-5906

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1. Scope

The AlerTox ELISA Soy is designed for the determination of soy in food, based on an antibody against soy trypsin inhibitor. The present report describes the validation process and summarizes 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 Soy

Replicate	Level 1	Level 2	Level 3	
1	45	247	478	
2	48	252	489	
3	42	250	497	
4	45	240	399	
5	45	260	499	
6	43	241	483	
7	48	267	463	
8	48	221	562	
9	45	261	537	
10	44	254	500	
11	42	258	505	
12	38	277	466	
13	47	276	457	
14	48	257	482	
15	46	272	447	
16	44	244	450	
17	49	244	475	
18	48	241	458	
19	50	236	472	
20	43	262	389	
Mean	45	253	475	
SD	3.0	14.3	39.6	RMS
CV [%]	6.5	5.7	8.3	6.9

The coefficient of variation is ranging from 5.7% to 8.3% 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 Soy

Assay No.	Level 1	Level 2	Level 3	
1	44	230	398	
2	44	234	421	
3	34	256	434	
4	47	235	372	
Mean	42	239	406	
SD	5.6	11.8	27.5	RMS
CV [%]	13.2	5.0	6.8	9.0

The coefficient of variation is ranging from 5.0% to 13.2% depending on the concentration.

3. Recovery

For recovery experiments different sample matrices were spiked with soy trypsin inhibitor 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 Soy

Cornflakes 1		
Target Value	Actual Concentration	Recovery [%]
100 ppb	94	94
400 ppb	456	114
	Mean	104

Cornflakes 2		
Target Value	Actual Concentration	Recovery [%]
100 ppb	95	95
400 ppb	393	98
	Mean	96

Cornflakes	Mean	100
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Cookie

Target Value	Actual Concentration	Recovery [%]
100 ppb	107	107
400 ppb	415	104
	Mean	106

Ice cream

Target Value	Actual Concentration	Recovery [%]
100 ppb	74	74
400 ppb	323	81
	Mean	77

Instant soup

Target Value	Actual Concentration	Recovery [%]
100 ppb	95	95
400 ppb	342	86
	Mean	90

Sausage 1

Target Value	Actual Concentration	Recovery [%]
200 ppb	254	127
400 ppb	470	117
Sausage 2	Mean	122

Target Value	Actual Concentration	Recovery [%]
200 ppb	217	108
400 ppb	378	94
	Mean	101

Sausage 3

Target Value	Actual Concentration	Recovery [%]
200 ppb	126	63
400 ppb	268	67
	Mean	65

Sausage 4

Target Value	Actual Concentration	Recovery [%]
200 ppb	206	103
400 ppb	347	87
	Mean	95

Sausages	Mean	96
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Mean recoveries are ranging from 77% 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 $OD_{\text{mean}} + 3x$ 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 Soy

Replicate	Sample diluent [OD]
1	0.193
2	0.175
3	0.176
4	0.190
5	0.178
6	0.172
7	0.190
8	0.189
9	0.184
10	0.159
11	0.184
12	0.267
13	0.239
14	0.170
15	0.206
16	0.192
17	0.189
18	0.167
19	0.166
20	0.202
21	0.183
22	0.180
23	0.170
24	0.177
Mean	0.187
SD	0.024
Limit of Detection	16 ppb

The limit of detection is 16 ppb of soy trypsin inhibitor. The lowest positive standard (50 ppb) was defined as limit of quantification (LOQ).

5. Linearity

Linearity was determined by spiking cornflakes, cookies, ice cream, chocolate, instant soup and sausage samples with soy trypsin inhibitor 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 Soy

Cornflakes

Target Value	Concentration [ppm]	Recovery [%]
1000 ppb	1105	100
500 ppb	753	136
250 ppb	360	130
125 ppb	182	132
63 ppb	79	114
	Mean [%]	128

Cookies

Target Value	Concentration [ppm]	Recovery [%]
1000 ppb	1108	100
500 ppb	653	118
250 ppb	287	104
125 ppb	145	105
63 ppb	50	72
	Mean [%]	100

Ice cream

Target Value	Concentration [ppm]	Recovery [%]
1000 ppb	825	100
500 ppb	418	101
250 ppb	249	121
125 ppb	103	100
63 ppb	51	99
	Mean [%]	105

Chocolate

Target Value	Concentration [ppm]	Recovery [%]
1000 ppb	837	100
500 ppb	395	94
250 ppb	231	110
125 ppb	95	91
63 ppb	48	92
	Mean [%]	97

Instant soup

Target Value	Concentration [ppm]	Recovery [%]
1000 ppb	722	100
500 ppb	396	110
250 ppb	230	127
125 ppb	97	107
63 ppb	51	113
	Mean [%]	114

Sausage

Target Value	Concentration [ppm]	Recovery [%]
1000 ppb	937	100
500 ppb	409	87
250 ppb	216	92
125 ppb	86	73
63 ppb	42	72
	Mean [%]	81

For different matrices the mean linearity is ranging from 81% to 128%. 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 Soy

Wheat	Peanut
Rye	Hazelnut
Barley	Almond
Oats	Bean
Milk	Gelatin (pork)
Cocoa	Beef
Pork meat	Chicken
Rice	Sucrose
Corn	Egg
Pea	

The following cross-reactivities could be determined:

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

Food	Cross-reactivity [%]
Sesame	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 un-spiked cookie sample and a sample spiked with 400 ppb soy trypsin inhibitor 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 Soy

Sample	Result 60 °C	Result 25 °C	Result 40 °C	Result 70 °C
Cookie 0 ppb	< 40 ppb	< 40 ppb	< 40 ppb	< 40 ppb
Cookie 400 ppb	393 ppb	357 ppb	423 ppb	418 ppb

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 Soy

Sample	Result 15 min	Result 10 min	Result 20 min
Cookie 0 ppb	< 40 ppb	< 40 ppb	< 40 ppb
Cookie 400 ppb	383 ppb	391 ppb	458 ppb

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 Soy

Sample	Result 20 min	Result 16 min	Result 24 min
Cookie 0 ppb	< 40 ppb	< 40 ppb	< 40 ppb
Cookie 100 ppb	101 ppb	83 ppb	141 ppb

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.

8. Application for soy products

For the calculation of the corresponding concentrations of processed soy products the amount of determined soy trypsin inhibitor has to be multiplied with a product specific conversion factor. Product specific conversion factors were determined by extraction, serial dilution and determination of the corresponding soy trypsin inhibitor concentration. The following conversion factors have been determined:

Table 11: Conversion factors for the determined STI contents

Soy product	Conversion factor
Soy flour, roasted	476
Soy flour, unroasted	42.6
Soy protein, roasted	190
Soy protein, unroasted	17
Soy milk	2500
Tofu	50000

Please note:

Soy sauce gives no positive results due to the complete destruction of the soy protein structure by either conventional fermentation or by hydrolysis for industrial made products!

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