AlerTox® ELISA Soy Kit
Claim Support Summary

REF KIT3047

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1. Scope

The AlerTox® ELISA Soy Kit is based on an antibody against soy trypsin inhibitor (STI) and is designed to determine soy content in food. **This report describes the validation process and its results for kits with lot number 340225 and later.** For reports for kits with earlier lot numbers, contact us at www.hygiena.com/support.

Definitions

CV = Coefficient of variation

LOD = Limit of detection

LOQ = Limit of quantification

OD = Optical density

ppb = µg of STI protein per kg or L of sample (µg/kg or µg/L)

SD = Standard deviation

2. Precision

2.1 Intra-Assay Variation

The intra-assay variation (Repeatability) was determined by testing a control at 3 concentrations in 20-fold replicates (Table 1).

Table 1. AlerTox ELISA Soy Kit Intra-Assay Variation.

Replicate	Concentration 1	Concentration 2	Concentration 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	**Mean**
CV [%]	**6.5**	**5.7**	**8.3**	**6.8**

The coefficient of variation (CV) ranged from 5.7 to 8.3%, depending on the concentration tested.

2.2 Inter-Assay Variation

The inter-assay variation (Reproducibility) was determined by testing a controls at 3 concentrations in 4 different test runs of the same kit lot (Table 2).

Table 2. AlerTox ELISA Soy Kit Inter-Assay Variation.

Assay Number	Concentration 1	Concentration 2	Concentration 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	Mean
CV [%]	13.2	5.0	6.8	8.3

The CV ranged from 5.0 to 13.2%, depending on the concentration tested.

3. Analytical Sensitivity

To determine the analytical sensitivity of the AlerTox ELISA Soy Kit, sample diluent was assayed in 24-fold replicates. After identification of possible outliers, the mean and standard deviation of the optical density (OD) were calculated. The corresponding concentration of the mean OD + 3X standard deviation (SD) was defined as the limit of detection (LOD) (Table 3).

Table 3. Matrix-Independent Analytical Sensitivity of the AlerTox ELISA Soy Kit.

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
LOD	**16 ppb**

LOD is 16 ppb of soy trypsin inhibitor.

The lowest positive standard (50 ppb) was defined as the limit of quantification (LOQ) to ensure that measurements of important, uncontaminated matrices (e.g., milk, wheat, rye, oats, barley, cocoa and pork) result in concentrations lower than this value.

4. Recovery

For recovery experiments, different sample matrices were spiked with soy trypsin inhibitor at 2 final concentrations (100 or 200 and 400 ppb) after performing all sample pre-treatment steps. The tested samples and calculated results are shown in Table 4.

Table 4. Recovery of Various Samples Tested with the AlerTox ELISA Soy Kit.

Cornflakes, Type 1

Spiked Value	Measured Concentration [ppb]	Recovery [%]
100 ppb	94	94
400 ppb	456	114
	Mean	**104**

Cornflakes, Type 2

Spiked Value	Measured Concentration [ppb]	Recovery [%]
100 ppb	95	95
400 ppb	393	98
	Mean	**96**

Cookie

Spiked Value	Measured Concentration [ppb]	Recovery [%]
100 ppb	107	107
400 ppb	415	104
	Mean	**106**

Ice Cream

Spiked Value	Measured Concentration [ppb]	Recovery [%]
100 ppb	74	74
400 ppb	323	81
	Mean	**77**

Chocolate

Spiked Value	Measured Concentration [ppb]	Recovery [%]
100 ppb	71	71
400 ppb	336	84
	Mean	**77**

Instant Soup

Spiked Value	Measured Concentration [ppb]	Recovery [%]
100 ppb	95	95
400 ppb	342	86
	Mean	**90**

Sausage, Type 1

Spiked Value	Measured Concentration [ppb]	Recovery [%]
200 ppb	254	127
400 ppb	470	117
Mean		**122**

Sausage, Type 2

Spiked Value	Measured Concentration [ppb]	Recovery [%]
200 ppb	217	108
400 ppb	378	94
Mean		**101**

Sausage, Type 3

Spiked Value	Measured Concentration [ppb]	Recovery [%]
200 ppb	126	63
400 ppb	268	67
Mean		**65**

Sausage, Type 4

Spiked Value	Measured Concentration [ppb]	Recovery [%]
200 ppb	206	103
400 ppb	347	87
Mean		**95**

Mean recoveries ranged from 65 to 122%, depending on the sample matrix.

5. Linearity

Linearity was determined by spiking cornflakes, cookies, ice cream, chocolate, instant soup and sausage samples with soy trypsin inhibitor and testing serial dilutions (1,000 to 62.5 ppb) of the resulting matrices (Table 5). For calculating linearity, the highest concentration was defined as the reference value (100%), and further dilutions were expressed as a percent of this reference after accounting for the dilution factor.

Table 5. Matrix-Dependent Linearity of the AlerTox ELISA Soy Kit.

Cornflakes

Spiked Value	Measured Concentration [ppb]	Recovery [%]
1,000 ppb	1,105	Reference* (100)
500 ppb	753	136
250 ppb	360	130
125 ppb	182	132
62.5 ppb	79	114
Mean		**128**

* The highest concentration was defined as the reference value (100%). Further dilutions were expressed as a percent of this reference after accounting for the dilution factor.

Cookies

Spiked Value	Measured Concentration [ppb]	Recovery [%]
1,000 ppb	1,108	Reference* (100)
500 ppb	653	118
250 ppb	287	104
125 ppb	145	105
62.5 ppb	50	72
	Mean	100

Ice Cream

Spiked Value	Measured Concentration [ppb]	Recovery [%]
1,000 ppb	825	Reference* (100)
500 ppb	418	101
250 ppb	249	121
125 ppb	103	100
62.5 ppb	51	99
	Mean	105

Chocolate

Spiked Value	Measured Concentration [ppb]	Recovery [%]
1,000 ppb	837	Reference* (100)
500 ppb	395	94
250 ppb	231	110
125 ppb	95	91
62.5 ppb	48	92
	Mean	97

Instant Soup

Spiked Value	Measured Concentration [ppb]	Recovery [%]
1,000 ppb	722	Reference* (100)
500 ppb	396	110
250 ppb	230	127
125 ppb	97	107
62.5 ppb	51	113
	Mean	114

Sausage

Spiked Value	Measured Concentration [ppb]	Recovery [%]
1,000 ppb	937	Reference* (100)
500 ppb	409	87
250 ppb	216	92
125 ppb	86	73
62.5 ppb	42	72
	Mean	81

* The highest concentration was defined as the reference value (100%). Further dilutions were expressed as a percent of this reference after accounting for the dilution factor.

For different matrices, the mean linearity ranged from 81 to 128%. The linearity was independent of the specific concentration and may have been affected by the precision, as stated in Section 2.

6. Cross-Reactivity

No cross-reactivity is defined as a result that is less than the LOQ. For the AlerTox ELISA Soy Kit, the LOQ is 50 ppb STI. During testing, no cross-reactivity was detected in the foods included in Table 6.

Table 6. Non-Cross-Reactive Food Matrices with the AlerTox ELISA Soy Kit.

Raw Material	Concentration [ppb]	Raw Material	Concentration [ppb]	Raw Material	Concentration [ppb]
Almond	0.0	Dill	0.4	Paprika	0.3
Apricot	0.0	Duck	2.2	Pea	5.9
Barley	0.0	Egg	0.0	Peach	1.0
Bean, white	10.0	Fennel	0.1	Peanut	0.4
Beef (cooked)	0.7	Flaxseed	11.5	Pecan	0.0
Beef (raw)	0.0	Garden cress	0.4	Pepper, black	1.7
Brazil nut	0.0	Garlic (fresh)	0.8	Pine seed	3.0
Buckwheat	1.7	Garlic (granulated)	0.0	Pistachio	4.0
Cabbage, white	0.0	Gelatin, cow	1.1	Plum	0.0
Caraway	0.4	Gelatin, fish	0.0	Poppy seed	0.6
Cardamom	0.0	Ginger (fresh)	0.6	Pork	1.1
Carob gum	1.1	Ginger (ground)	0.0	Potato	0.0
Carrot	0.0	Gliadin	5.6	Prawn (cooked)	0.3
Cashew	0.0	Guar gum	4.2	Prawn (raw)	1.7
Cayenne	2.8	Gum arabic	0.0	Pumpkin seed	9.2
Celery	0.0	Hazelnut	0.1	Radish	11.3
Cherry	1.0	Horseradish	0.8	Rapeseed	0.0
Chervil	0.0	Isinglass	3.8	Rice	0.0
Chestnut	0.8	Kidney bean	21.5	Rye	7.3
Chia	0.4	Kiwi	0.0	Saccharose	0.0
Chicken	0.0	Lamb	2.9	Sesame	1.0
Chickpea	7.1	Leek	1.9	Shrimps	0.0
Chili	1.9	Lentil	2.2	Split pea	3.9
Cinnamon	7.5	Lupine	3.7	Sunflower seeds	0.0
Clove	7.6	Macadamia	0.0	Thyme	0.4
Cocoa	4.2	Milk, cow	2.2	Tomato	0.0
Coconut	1.7	Milk, goat	1.0	Turkey	3.5
Cod	0.6	Milk, sheep	0.0	Turmeric	0.4
Corn	1.7	Mustard	1.7	Walnut	0.0
Crab (cooked)	0.0	Nutmeg	0.0	Wheat	0.1
Crab (raw)	0.0	Oats	0.8		
Cumin	0.0	Onion	1.9		

The following cross-reactivities were determined (Table 7):

Table 7. Cross-Reactive Food Matrices with the AlerTox ELISA Soy Kit.

Cross-Reactive Food Matrices		
Food	**Concentration [ppb]**	**Cross-reactivity [%]**
Adzuki bean	135	0.00001
Fenugreek	53	0.000005

7. Robustness

Robustness was determined by the variation of different handling parameters defined in the instruction manual. The results obtained under various conditions were compared to the results obtained by following the instruction manual. An unspiked cookie sample and a sample spiked with soy trypsin inhibitor were analyzed.

7.1 Variation of Extraction Temperature

The extraction temperature, defined as 60 °C, was changed to 25 °C, 40 °C and 70 °C (Table 8).

Table 8. Variation of Extraction Temperature with the AlerTox ELISA Soy Kit.

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

Considering the precision data (Section 2), the results did *not* differ significantly.

7.2 Variation of Extraction Time

The extraction time, defined as 15 min, was changed to 10 min and 20 min (Table 9).

Table 9. Variation of Extraction Time with the AlerTox ELISA Soy Kit.

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

Considering the precision data (Section 2), the results did *not* differ significantly.

7.3 Drift

In contrast to the test procedure 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).

Table 10. Drift in the AlerTox ELISA Soy Kit.

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

The results differed significantly. Drift in extensive test runs should be avoided by pipetting calibrators once before and once after the samples and using the mean value for the calculation.

8. Application for Soy Products

To calculate the corresponding concentrations of processed soy products, the determined amount of soy trypsin inhibitor is multiplied by product-specific conversion factors (Table 11), which were determined by extraction, serial dilution and determination of the corresponding soy trypsin inhibitor concentration.

Table 11. Drift in the AlerTox ELISA Soy Kit.

Soy Product	Conversion Factor (Multiply by)
Soy flour (roasted)	250
Soy flour (unroasted)	52
Soy protein isolate (90%)	440
Soy milk	3,700
Textured soy (granulated)	4,550
Tofu	7,000

9. Summary

- The AlerTox ELISA Soy Kit is sensitive with an LOD of 16 ppb (µg of STI per kg or L of sample) and an LOQ of 50 ppb of STI (µg/kg or µg/L).

- The AlerTox ELISA Soy Kit provides consistent, precise results, as indicated by results of the intra- and inter-assay studies (mean CV < 10%, Section 2).

- Robust recovery (Section 4) and linearity (Section 5) mean that samples and their dilutions are quantitated accurately.

- Extensive cross-reactivity studies showed high specificity (Section 6), except for low cross-reactivity with adzuki bean and fenugreek.

- Drift in large test runs can be avoided by pipetting calibrators once before and once after the samples and using the calibrator mean values for the calculations.