

# AlerTox<sup>®</sup> ELISA Sesame KIT3051

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## 1. Scope

The AlerTox® ELISA Sesame Kit is designed for the determination of sesame in food. This report describes the validation process and its results.

## 2. Precision

### 2.1 Intra-Assay Variation

The intra-assay variation (Repeatability) was determined by testing three controls of various concentration levels in 20-fold replicates.

**Table 1. Intra-Assay Variation of the AlerTox ELISA Sesame Kit.**

Replicate	Concentration Level 1	Concentration Level 2	Concentration Level 3	
1	5.73	17.6	42.4	
2	5.45	17.6	30.8	
3	5.43	16.1	32.9	
4	5.26	15.9	43.5	
5	5.77	16.7	40.2	
6	5.29	19.1	43.1	
7	5.36	18.1	36.4	
8	5.31	17.4	39.2	
9	5.94	16.2	32.4	
10	4.84	16.1	36.1	
11	5.24	17.8	36.0	
12	5.27	18.4	29.7	
13	5.08	16.1	38.0	
14	5.72	17.5	29.0	
15	5.29	15.2	39.6	
16	5.21	14.5	35.4	
17	5.30	17.4	33.1	
18	5.86	17.5	33.9	
19	5.37	16.7	39.2	
20	4.99	16.8	29.0	
Mean	5.38	16.9	36.0	
SD*	0.29	1.11	4.60	Mean
CV† [%]	5.4	6.5	12.8	8.2

\* SD = standard deviation

† CV = coefficient of variation

The coefficient of variation (CV) ranged from 5.4 to 12.8%, depending on the concentration tested.

## 2.2 Inter-Assay Variation

The inter-assay variation (Reproducibility) 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 Sesame Kit.**

Assay Number	Concentration Level 1	Concentration Level 2	Concentration Level 3	
1	6.34	20.4	31.8	
2	5.52	17.0	33.1	
3	5.53	17.6	32.6	
4	6.12	16.4	34.6	
Mean	5.88	17.8	33.1	
SD*	0.42	1.77	1.18	Mean
CV† [%]	<b>7.1</b>	<b>9.9</b>	<b>3.6</b>	<b>6.9</b>

\* SD = standard deviation

† CV = coefficient of variation

The coefficient of variation (CV) ranged from 3.6 to 9.9%, depending on the concentration tested.

### 3. Analytical Sensitivity

For determination of the analytical sensitivity, sample diluent and sesame-free soup, ice-cream, sausage, salad sauce and cracker samples were assayed in 24-fold replicates. After identification of possible outliers, the mean and standard deviation of the OD were calculated. The corresponding concentration of the mean OD + 3X standard deviation was defined as the limit of detection (LOD) (Tables 3).

**Table 3. Matrix-Independent and Matrix-Dependent Analytical Sensitivity of the AlerTox ELISA Sesame Kit.**

Replicate	Sample Diluent [OD]	Biscuit Matrix [OD]	Ice Cream Matrix [OD]	Sausage Matrix [OD]	Salad Sauce Matrix [OD]	Cracker Matrix [OD]
1	0.132	0.110	0.122	0.139	0.105	0.073
2	0.140	0.096	0.119	0.100	0.090	0.071
3	0.114	0.099	0.109	0.116	0.090	0.065
4	0.120	0.110	0.109	0.120	0.126	0.072
5	0.134	0.113	outlier	0.124	0.115	0.079
6	0.135	0.111	0.107	0.122	0.129	0.073
7	0.158	0.113	0.104	0.133	0.099	0.073
8	0.133	0.119	0.110	0.118	0.094	0.075
9	0.128	0.106	0.122	0.123	0.106	0.068
10	0.128	0.106	0.118	0.118	0.087	0.062
11	0.168	0.100	0.101	0.118	0.084	0.059
12	0.122	0.110	0.113	0.118	0.096	0.064
13	0.137	0.107	0.110	0.118	0.093	0.066
14	0.146	0.107	0.113	0.126	outlier	0.064
15	0.126	0.111	0.107	0.116	0.083	0.070
16	0.163	0.103	0.106	0.116	0.087	0.071
17	0.127	0.114	0.127	0.127	0.095	0.074
18	0.139	0.104	0.120	0.104	0.112	0.065
19	0.150	0.101	0.118	0.108	0.093	0.063
20	0.165	outlier	0.111	0.115	0.097	0.070
21	0.157	0.118	0.108	0.113	0.093	0.068
22	0.183	0.122	0.108	0.120	0.091	0.063
23	0.133	0.121	0.117	0.115	0.092	0.067
24	0.133	0.110	0.107	0.108	0.093	0.071
Mean	0.140	0.109	0.112	0.118	0.098	0.069
SD*	0.017	0.007	0.007	0.009	0.012	0.005
LOD <sup>†</sup>	<b>0.17 ppm</b>	<b>0.12 ppm</b>	<b>0.01 ppm</b>	<b>0.06 ppm</b>	<b>0.04 ppm</b>	<b>0.01 ppm</b>

\* SD = standard deviation

† LOD = limit of detection



The limits of detection varied from 0.01 to 0.17 ppm based on the sample matrix. Note that the derived limits of detection were strictly dependent on the coefficient of variation and thus, may vary in each individual test. The data for sample diluent and matrices were not determined in the same test runs.

The lowest positive standard (2 ppm) was defined as the limit of quantification (LOQ) to ensure that all uncontaminated matrices (e.g., milk, egg, wheat, rye, oats and barley) result in concentrations lower than this value.

## 4. Recovery

For recovery experiments, different sample matrices were spiked with sesame to obtain a range of final concentrations after performing all sample pre-treatment steps. Tested samples and results are shown in Table 4.

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

<b>Soup</b>		
<b>Spiked Value</b>	<b>Measured Concentration [ppm]</b>	<b>Recovery [%]</b>
5 ppm	5.41	108
15 ppm	19.5	130
40 ppm	36.7	92
	Mean	110

  

<b>Ice Cream</b>		
<b>Spiked Value</b>	<b>Measured Concentration [ppm]</b>	<b>Recovery [%]</b>
5 ppm	4.75	95
15 ppm	13.4	89
40 ppm	28.1	70
	Mean	85

  

<b>Sausage</b>		
<b>Spiked Value</b>	<b>Measured Concentration [ppm]</b>	<b>Recovery [%]</b>
5 ppm	4.12	82
15 ppm	15.0	100
40 ppm	37.2	93
	Mean	92

  

<b>Salad Sauce</b>		
<b>Spiked Value</b>	<b>Measured Concentration [ppm]</b>	<b>Recovery [%]</b>
5 ppm	4.99	100
15 ppm	16.4	109
40 ppm	28.2	71
	Mean	93

**Cracker**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
5 ppm	6.22	124
15 ppm	14.2	94
40 ppm	43.9	110
	Mean	109

Mean recoveries ranged from 85 to 110%, depending on the sample matrix.

## 5. Linearity

Linearity was determined by spiking soup, ice-cream, sausage, salad sauce and cracker samples with sesame and testing subsequent dilutions of the resulting extracts (Table 5). For calculation of the linearity, the highest concentration was defined as the reference value (100%), and further dilutions were expressed as the percentage of this reference value after considering the dilution factor.

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

**Soup**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
60 ppm	59.4	100
30 ppm	21.7	73
15 ppm	12.4	83
7.5 ppm	6.12	82
3.75 ppm	3.25	88
	Mean	82

**Ice Cream**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
60 ppm	64.0	100
30 ppm	29.4	92
15 ppm	17.6	110
7.5 ppm	9.95	124
3.75 ppm	5.17	129
	Mean	114

**Sausage**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
60 ppm	60.5	100
30 ppm	24.9	82
15 ppm	14.2	94
7.5 ppm	7.39	98
3.75 ppm	4.03	107
	Mean	95

**Salad Sauce**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
60 ppm	49.9	100
30 ppm	27.6	111
15 ppm	15.2	122
7.5 ppm	7.78	125
3.75 ppm	3.85	123
	Mean	120

**Cracker**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
60 ppm	30.12	100
30 ppm	11.14	74
15 ppm	6.89	92
7.5 ppm	4.09	109
3.75 ppm	2.32	123
	Mean	99

For different matrices, the mean linearity ranged from 82 to 120%. The linearity was independent of the specific sesame concentration and may have been affected by the intra-assay and inter-assay variation, as stated in Section 2.



## 6. Cross-Reactivity

No cross-reactivity is defined as a result less than the LOQ. For the AlerTox ELISA Sesame kit, the LOQ is 2.0 ppm. During testing, no cross-reactivity was detected in the following foods:

**Table 6. Non-Cross-Reactive Food Matrices in the AlerTox ELISA Sesame Kit.**

Non-Cross-Reactive Matrices			
Adzuki bean	Almond	Apricot	Barley
Bean white	Beef	Beef (cooked)	Brazil nut
Buckwheat	Cabbage white	Caraway	Cardamom
Carob gum	Carrot	Cashew	Cayenne
Celery	Cherry	Chervil	Chestnut
Chia seeds	Chicken	Chickpea	Chili
Cinnamon	Clove	Cocoa	Coconut
Cod	Corn	Cumin	Cumin black
Dill	Duck	Egg dried	Fennel
Fenugreek	Flaxseed	Garden cress	Garlic (fresh)
Garlic (granulated)	Gelatin cow	Gelatin fish	Ginger (fresh)
Ginger (ground)	Gliadin	Guar gum	Gum arabic
Hazelnut	Horseradish	Isinglass	Kidney bean
Kiwi	Lamb	Leek	Lentil
Lupin	Macadamia	Milk cow	Milk goat
Mustard yellow	Nutmeg	Oats	Onion
Paprika	Pea	Peach	Peanut
Pecan	Pepper black	Pine seed	Pistachio
Plum	Poppy seed	Pork	Potato
Prawn (cooked)	Prawn (raw)	Pumpkin seed	Radish
Rice	Rye	Saccharose	Shrimp (cooked)
Shrimp (raw)	Soy flour	Soy lecithin	Split pea
Sunflower seed	Thyme	Tofu	Tomato
Turkey	Turmeric	Walnut	Wheat

The following cross-reactivities were determined:

**Table 7. Cross-Reactive Food Matrices in the AlerTox ELISA Sesame Kit.**

Raw material	Cross-reactivity [%]
Black Sesame	16
Psyllium Husk	0.001
Rapeseed	0.0003





## 7. Robustness

Robustness was determined by varying some of the 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 soup sample and a sample spiked with 15 ppm sesame 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. Variation of Extraction Temperature in the AlerTox ELISA Sesame Kit.**

Sample	Result 60 °C	Result 25 °C	Result 40 °C	Result 70 °C
Soup, 0 ppm	0.82 ppm	0.94 ppm	0.83 ppm	0.81 ppm
Soup, 15 ppm	13.3 ppm	10.5 ppm	12.4 ppm	14.3 ppm

Considering the intra- and inter-assay variations (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. Variation of Extraction Time in the AlerTox ELISA Sesame Kit.**

Sample	Result 15 min	Result 10 min	Result 20 min
Soup, 0 ppm	0.82 ppm	1.00 ppm	0.91 ppm
Soup, 15 ppm	13.3 ppm	12.6 ppm	11.5 ppm

Considering the intra- and inter-assay variation (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 5 minutes compared to the calibrators (20 min).

**Table 10. Drift in the AlerTox ELISA Sesame Kit.**

Sample	Result 20 min	Result 15 min	Result 25 min
Soup, 0 ppm	0.82 ppm	0.25 ppm	1.00 ppm
Soup, 15 ppm	13.3 ppm	8.23 ppm	16.4 ppm

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.