

# Validation Report

## **AlerTox ELISA Mustard** KIT3058/KT-5915

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## 1. Scope

The AlerTox ELISA Mustard is designed for the determination of Mustard 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 Mustard*

Replicate	Level 1	Level 2	Level 3	
1	3.4	11.5	28	
2	3.8	12.8	32	
3	4.1	11.8	31	
4	4.7	12.2	32	
5	4.6	12.1	30	
6	4.3	12.2	31	
7	4.6	11.0	30	
8	4.6	13.5	30	
9	3.6	12.4	31	
10	3.8	12.5	29	
11	4.0	11.2	31	
12	4.3	12.3	31	
13	3.9	12.6	32	
14	3.8	12.3	29	
15	3.4	12.2	28	
16	4.2	13.2	33	
17	2.9	12.7	28	
18	3.5	12.2	32	
19	3.6	13.2	32	
20	5.1	13.6	35	
<b>Mean</b>	4.0	12.4	31	
<b>SD</b>	0.53	0.70	1.9	<b>RMS</b>
<b>CV [%]</b>	<b>13.3</b>	<b>5.7</b>	<b>6.0</b>	<b>9.0</b>

The coefficient of variation is ranging from 5.7% to 13.3% 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 Mustard*

Assay No.	Level 1	Level 2	Level 3	
<b>1</b>	5.5	13.2	29	
<b>2</b>	4.7	17.2	30	
<b>3</b>	5.0	15.1	35	
<b>4</b>	4.4	20.0	34	
<b>Mean</b>	4.9	16.4	32	
<b>SD</b>	0.47	2.91	3.0	<b>RMS</b>
<b>CV [%]</b>	<b>9.6</b>	<b>17.8</b>	<b>9.4</b>	<b>12.9</b>

The coefficient of variation is ranging from 9.4% to 17.8% depending on the concentration.

## 3. Recovery

For recovery experiments different sample matrices were spiked with mustard 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 Mustard*

### ***Canned Soup***

Target Value	Actual Concentration	Recovery [%]
<b>5 ppm</b>	5.5	110
<b>15 ppm</b>	13.4	89
<b>35 ppm</b>	30.9	88
	<b>Mean</b>	<b>96</b>

### ***Salad Sauce***

Target Value	Actual Concentration	Recovery [%]
<b>5 ppm</b>	4.1	81
<b>15 ppm</b>	10.2	68
<b>35 ppm</b>	28.0	80
	<b>Mean</b>	<b>76</b>

***Cheese***

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.3	86
15 ppm	11.7	78
35 ppm	35.8	102
	<b>Mean</b>	<b>89</b>

***Sausage***

Target Value	Actual Concentration	Recovery [%]
5 ppm	5.1	102
15 ppm	14.9	99
35 ppm	32.7	93
	<b>Mean</b>	<b>98</b>

***Instant Soup***

Target Value	Actual Concentration	Recovery [%]
5 ppm	5.0	101
15 ppm	10.6	71
35 ppm	24.2	69
	<b>Mean</b>	<b>80</b>

***Mixed Herbs***

Target Value	Actual Concentration	Recovery [%]
5 ppm	3.9	77
15 ppm	11.3	75
35 ppm	28.7	82
	<b>Mean</b>	<b>78</b>

Mean recoveries are ranging from 76% 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 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 Mustard*

<b>Replicate</b>	<b>Sample diluent [OD]</b>
1	0.214
2	0.212
3	0.210
4	0.198
5	0.191
6	0.185
7	0.186
8	0.185
9	0.214
10	0.189
11	0.193
12	0.170
13	0.187
14	0.176
15	0.169
16	0.157
17	0.204
18	0.209
19	0.198
20	0.179
21	0.197
22	0.181
23	0.171
24	0.166
<b>Mean</b>	<b>0.189</b>
<b>SD</b>	<b>0.016</b>
<b>Limit of Detection</b>	<b>1.3 ppm</b>

The limit of detection is 1.3 ppm of mustard. The lowest positive standard (2 ppm) was defined as limit of quantification (LOQ).

## 5. Linearity

Linearity was determined by spiking sausage, salad sauce, canned soup, instant soup, cheese and mixed herbs samples with mustard 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 percent of this reference after consideration of the dilution factor

Table 5: Matrix dependent linearity of the AlerTox ELISA Mustard

### ***Canned Soup***

<b>Target Value</b>	<b>Concentration [ppm]</b>	<b>Recovery [%]</b>
<b>60 ppm</b>	60.1	100
<b>30 ppm</b>	31.7	105
<b>15 ppm</b>	15.1	100
<b>7.5 ppm</b>	7.2	96
<b>3.75 ppm</b>	3.5	93
	<b>Mean [%]</b>	<b>99</b>

### ***Salad Sauce***

<b>Target Value</b>	<b>Concentration [ppm]</b>	<b>Recovery [%]</b>
<b>60 ppm</b>	43.8	100
<b>30 ppm</b>	21.2	97
<b>15 ppm</b>	11.2	102
<b>7.5 ppm</b>	5.7	104
<b>3.75 ppm</b>	2.5	91
	<b>Mean [%]</b>	<b>99</b>

### ***Sausage***

<b>Target Value</b>	<b>Concentration [ppm]</b>	<b>Recovery [%]</b>
<b>60 ppm</b>	55.6	100
<b>30 ppm</b>	26.3	95
<b>15 ppm</b>	13.0	94
<b>7.5 ppm</b>	6.3	91
<b>3.75 ppm</b>	3.1	89
	<b>Mean [%]</b>	<b>92</b>

### ***Cheese***

<b>Target Value</b>	<b>Concentration [ppm]</b>	<b>Recovery [%]</b>
<b>60 ppm</b>	55.7	100
<b>30 ppm</b>	27.7	99
<b>15 ppm</b>	12.2	88
<b>7.5 ppm</b>	6.9	99
<b>3.75 ppm</b>	2.8	80
	<b>Mean [%]</b>	<b>92</b>

### ***Instant Soup***

<b>Target Value</b>	<b>Concentration [ppm]</b>	<b>Recovery [%]</b>
<b>60 ppm</b>	43.7	100
<b>30 ppm</b>	23.5	108
<b>15 ppm</b>	12.8	117
<b>7.5 ppm</b>	6.5	119
<b>3.75 ppm</b>	2.9	106
	<b>Mean [%]</b>	<b>113</b>

### ***Mixed Herbs***

<b>Target Value</b>	<b>Concentration [ppm]</b>	<b>Recovery [%]</b>
<b>60 ppm</b>	53.0	100
<b>30 ppm</b>	26.7	101
<b>15 ppm</b>	14.2	107
<b>7.5 ppm</b>	6.6	100
<b>3.75 ppm</b>	3.3	100
	<b>Mean [%]</b>	<b>102</b>

For different matrices the mean linearity is ranging from 92% to 113%. 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) could be detected:

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

Adzuki	Cow's milk	Peanut
Almond	Cumin	Pecan
Apricot	Curcuma	Pepper
Barley	Dill	Pine seed
Bean, white	Duck	Pistachio
Beef	Fennel	Poppy seed
Bovine gelatin	Fenugreek	Pork
Brazil nut	Garden cress	Potato
Buckwheat	Garlic	Pumpkin seed
Caraway	Gliadin	Radish
Cardamon	Goat's milk	Rice
Carob gum	Guar gum	Rye
Carrot	Hazelnut	Saccharose
Cashew	Horseradish	Sesame
Cayenne	Kidney bean	Shrimp, cooked
Celery	Kiwi	Shrimp, raw
Cherry	Lamb	Shrimps
Chestnut	Leek	Soy flour
Chia	Lentil	Soy lecithin
Chicken	Linseed	Split peas
Chickpea	Lupin	Sunflower seeds
Chili	Macadamia	Thyme
Cinnamon	Nutmeg	Tomato
Clove	Oats	Turkey
Cocoa	Onion	Walnut
Coconut	Paprika	Wheat
Cod	Pea	White cabbage
Corn	Peach	

The following cross-reactivity could be determined:

<b>Food</b>	<b>Cross-reactivity [%]</b>
Brown mustard (seed)	59
Black mustard (seed)	50
Charlock mustard	48
Rapeseed	59



## 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 soup sample and a sample spiked with 15 ppm of mustard were analyzed respectively.

### 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 Mustard*

Sample	Result 60 °C	Result 25 °C	Result 40 °C	Result 70 °C
Soup 0 ppm	0 ppm	0 ppm	0 ppm	0 ppm
Soup 15 ppm	11.9 ppm	12.0 ppm	11.4 ppm	11.2 ppm

Under consideration of the intra-assay and inter-assay variations as stated in chapter 2 the results do not differ significantly.

### Variation of extraction time

The extraction time, defined as 15 min, was changed to 5 min. 10 min and 20 min. respectively.

*Table 9: Variation of extraction time in the AlerTox ELISA Mustard*

Sample	Result 15 min	Result 10 min	Result 20 min
Soup 0 ppm	0 ppm	0 ppm	0 ppm
Soup 15 ppm	11.9 ppm	11.0 ppm	10.7 ppm

Under consideration of the intra-assay and inter-assay variation as stated in chapter 2, the results do not differ significantly.

### 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 Mustard*

Sample	Result 20 min	Result 16 min	Result 24 min
Soup 0 ppm	0 ppm	0 ppm	0.2 ppm
Soup 15 ppm	11.9 ppm	9.8 ppm	13.5 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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