

Validation Report

AlerTox ELISA Crustacean

KIT3059/KT-5903

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1. Scope

The AlerTox ELISA Crustacean is designed for the determination of crustacean tropomyosin 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 Crustacean

Replicate	Level 1	Level 2	Level 3	
1	37.6	91.6	365	
2	35.4	89.6	351	
3	32.6	79.5	332	
4	31.3	81.7	331	
5	32.1	78.5	344	
6	34.1	77.5	329	
7	31.8	85.5	321	
8	35.8	90.6	392	
9	36.2	91.7	366	
10	33.5	96.5	335	
11	32.5	79.0	328	
12	32.6	78.0	319	
13	33.0	76.3	303	
14	33.9	81.4	334	
15	33.3	93.7	328	
16	32.9	93.2	330	
17	30.9	79.5	318	
18	30.6	85.5	316	
19	31.7	88.5	365	
20	31.9	81.7	334	
Mean	33.2	85.0	337	
SD	1.85	6.42	21.4	RMS
CV [%]	5.6	7.6	6.4	6.6

The coefficient of variation is ranging from 5.6% to 7.6% 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 Crustacean

Assay No.	Level 1	Level 2	Level 3	
1	39.3	82.9	337	
2	36.1	71.8	361	
3	40.1	74.1	341	
4	30.6	87.5	321	
Mean	36.5	79.1	340	
SD	4.31	7.37	16.4	RMS
CV [%]	11.8	9.3	4.8	9.1

The coefficient of variation is ranging from 4.8% to 11.8% depending on the concentration.

3. Recovery

For recovery experiments different sample matrices were spiked with tropomyosin 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 Crustacean

Soy sauce

Target Value	Actual Concentration	Recovery [%]
40 ppb	33.3	83
100 ppb	77.4	77
400 ppb	368	92
	Mean	84

Instant soup

Target Value	Actual Concentration	Recovery [%]
40 ppb	34.1	85
100 ppb	94.8	95
400 ppb	391	98
	Mean	93

Cracker

Target Value	Actual Concentration	Recovery [%]
40 ppb	34.8	87
100 ppb	88.6	89
400 ppb	382	95
	Mean	90

Fish

Target Value	Actual Concentration	Recovery [%]
40 ppb	36.9	92
100 ppb	105.5	106
400 ppb	327	82
	Mean	93

Meat

Target Value	Actual Concentration	Recovery [%]
40 ppb	41.7	104
100 ppb	103.3	103
400 ppb	338	84
	Mean	97

Mean recoveries are ranging from 84% to 97% 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 limits of detection according to the following table:

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

Replicate	Sample diluent [OD]
1	0.056
2	0.056
3	0.052
4	0.061

Replicate	Sample diluent [OD]
5	0.055
6	0.054
7	0.052
8	0.059
9	0.056
10	0.056
11	0.058
12	0.059
13	0.054
14	0.051
15	0.058
16	0.056
17	0.055
18	0.054
19	0.055
20	0.051
21	0.051
22	0.053
23	0.054
24	0.054
Mean	0.055
SD	0.003
Limit of Detection	0.9 ppb

The limit of detection (LOD) is 1 ppb of tropomyosin. The lowest positive standard (20 ppb) was defined as limit of quantification (LOQ).

5. Linearity

Linearity was determined by spiking soy sauce, instant soup, cracker, fish and meat samples with tropomyosin 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 Crustacean

Soy sauce

Target Value	Concentration [ppb]	Recovery [%]
400 ppb	367.8	100
200 ppb	199.3	108
100 ppb	83.5	91
50 ppb	43.4	94
25 ppb	21.3	93
	Mean [%]	97

Instant soup

Target Value	Concentration [ppb]	Recovery [%]
400 ppb	390.7	100
200 ppb	208.8	107
100 ppb	85.8	88
50 ppb	43.0	88
25 ppb	20.7	85
	Mean [%]	92

Cracker

Target Value	Concentration [ppb]	Recovery [%]
400 ppb	381.5	100
200 ppb	204.8	107
100 ppb	82.7	87
50 ppb	35.3	74
25 ppb	17.8	75
	Mean [%]	86

Fish

Target Value	Concentration [ppb]	Recovery [%]
400 ppb	327.3	100
200 ppb	186.1	114
100 ppb	79.2	97
50 ppb	36.9	90
25 ppb	17.3	85
	Mean [%]	96

Meat

Target Value	Concentration [ppb]	Recovery [%]
400 ppb	332.5	100
200 ppb	181.2	109
100 ppb	76.1	92
50 ppb	34.7	84
25 ppb	17.9	86
	Mean [%]	93

For different matrices the mean linearity is ranging from 86% to 97%. 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 Crustacean

Wheat	Cod	Soy lecithin	Hazelnut
Rye	Pork	Carrot	Almond
Oats	Beef	Potato	Pecan
Barley	Chicken	Leek	Brazil nut
Rice	Soy	Sunflower seed	Coconut
Corn	Sesame	Pumpkin seed	Walnut
Buckwheat	Celery	Pine seed	Pistachio
Milk	Pea	Cashew	Macadamia nut
Egg	Bean	Peanut	Chestnut
Oyster			

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 soy sauce sample and a sample spiked with 40 ppb and 200 ppb of tropomyosin were analyzed respectively.

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 7: Drift in the AlerTox ELISA Crustacean

Sample	Result 20 min	Result 15 min	Result 25 min
Soy sauce 0 ppb	0 ppb	0 ppb	0 ppb
Soy sauce 40 ppb	29.0 ppb	18.9 ppb	37.2 ppb
Soy sauce 200 ppb	182 ppb	122 ppb	228 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 crustaceans

For the calculation of the corresponding concentrations of crustaceans (dry weight) the amount of determined tropomyosin has to be multiplied with a crustacean specific conversion factor. Crustacean specific conversion factors were determined by extraction, serial dilution and determination of the corresponding tropomyosin concentration. The following conversion factors have been determined:

Table 8: Conversion factors in the AlerTox ELISA Crustacean

Crustacean	Conversion factor
Black tiger prawns, raw	60
Black tiger prawns, cooked	260
Lobster, raw	290
Lobster, cooked	270
Crawfish, raw	50
Crawfish, cooked	490
Shrimp, raw	70
Shrimp, cooked	70
Crab, blanched	230
Crab, cooked	520

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