

Validation Report

AlerTox ELISA Fish

KIT3060/KT-5920

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1. Scope

The AlerTox ELISA Fish is designed for the determination of fish residues 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 based on measured ppm of the AlerTox ELISA Fish

Replicate	Level 1 [ppm]	Level 2 [ppm]	Level 3 [ppm]	
1	3.82	9.62	38.64	
2	4.17	11.07	40.27	
3	4.10	11.07	38.60	
4	3.79	11.86	39.96	
5	3.30	10.54	37.89	
6	3.49	9.70	39.88	
7	3.69	11.57	37.89	
8	3.66	11.53	39.17	
9	3.98	11.28	37.85	
10	4.51	11.49	40.54	
11	3.56	10.74	37.51	
12	3.76	11.20	41.18	
13	3.53	10.99	34.17	
14	4.25	10.08	34.01	
15	4.97	10.87	33.68	
16	4.82	10.21	36.82	
17	3.46	10.25	33.60	
18	3.95	9.35	34.55	
19	4.21	9.70	34.75	
20	3.40	10.21	35.04	
Mean	3.92	10.67	35.46	
SD	0.46	0.74	2.54	RMS
CV [%]	11.8	7.0	7.2	8.9

The coefficient of variation is ranging from 7.0% to 11.8% depending on the concentration.

RMS = Root Mean Square

Table 2: Intra-assay variation based on measured OD values of the AlerTox ELISA Fish

Replicate	Level 1 OD_{450nm}	Level 2 OD_{450nm}	Level 3 OD_{450nm}	
1	0.224	0.413	1.080	
2	0.234	0.413	1.116	
3	0.232	0.432	1.079	
4	0.223	0.400	1.112	
5	0.208	0.425	1.110	
6	0.214	0.424	1.062	
7	0.220	0.418	1.093	
8	0.219	0.423	1.061	
9	0.229	0.405	1.119	
10	0.243	0.416	1.077	
11	0.216	0.389	1.126	
12	0.222	0.411	0.972	
13	0.215	0.389	0.986	
14	0.236	0.370	0.960	
15	0.255	0.408	1.036	
16	0.251	0.392	0.958	
17	0.228	0.449	0.981	
18	0.235	0.393	0.986	
19	0.211	0.389	0.993	
20	0.198	0.392	1.033	
Mean	0.226	0.408	1.047	
SD	0.014	0.019	0.059	RMS
CV [%]	6.3	4.6	5.6	5.5

The coefficient of variation is ranging from 4.6% to 6.3% 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 3: Inter-assay variation based on measured ppm of the AlerTox ELISA Fish

Assay No.	Level 1 [ppm]	Level 2 [ppm]	Level 3 [ppm]	
1	8.28	33.43	75.84	
2	9.01	34.51	93.53	
3	10.34	33.70	78.96	
4	8.71	36.31	81.92	
Mean	9.09	34.48	82.56	
SD	0.89	1.30	7.72	RMS
CV [%]	9.8	3.8	9.4	8.1

The coefficient of variation is ranging from 3.8% to 9.8% depending on the concentration.

Table 4: Inter-assay variation based on measured OD values of the AlerTox ELISA Fish

Assay No.	Level 1 OD_{450nm}	Level 2 OD_{450nm}	Level 3 OD_{450nm}	
1	0.461	1.242	1.877	
2	0.501	1.147	1.888	
3	0.483	1.185	2.046	
4	0.499	1.231	1.943	
Mean	0.486	1.201	1.939	
SD	0.019	0.044	0.077	RMS
CV [%]	3.8	3.6	4.0	3.8

The coefficient of variation is ranging from 3.6% to 4.0% depending on the concentration.

3. Recovery

For recovery experiments different sample matrices were spiked with cashew to obtain various final concentrations after performing all sample pre-treatment steps. Tested samples and results were as follows.

Table 5: Recovery of various samples tested with the AlerTox ELISA Fish

Red Wine

Target Value	Actual Concentration	Recovery [%]
10 ppm	10.7	107
40 ppm	39.4	98
100 ppm	104.4	104
	Mean	103

Soup

Target Value	Actual Concentration	Recovery [%]
10 ppm	11.9	119
40 ppm	46.4	116
100 ppm	115.9	116
	Mean	117

Worcester Sauce

Target Value	Actual Concentration	Recovery [%]
10 ppm	11.5	115
40 ppm	41.3	103
100 ppm	116.8	117
	Mean	112

Asia Sauce

Target Value	Actual Concentration	Recovery [%]
10 ppm	11.2	112
40 ppm	37.5	94
100 ppm	103.3	103
	Mean	103

Cracker

Target Value	Actual Concentration	Recovery [%]
10 ppm	10.6	106
40 ppm	36.0	90
100 ppm	102.2	102
	Mean	99

Surimi

Target Value	Actual Concentration	Recovery [%]
10 ppm	11.1	111
40 ppm	48.4	121
100 ppm	110.2	110
	Mean	114

Spring Roll

Target Value	Actual Concentration	Recovery [%]
10 ppm	9.7	97
40 ppm	36.0	90
100 ppm	92.4	92
	Mean	93

Mean recoveries are ranging from 93% to 117% depending on the sample matrix.

4. Analytical Sensitivity

For determination of the analytical sensitivity sample diluent and cashew free cookies, cornflakes, ice-cream and dark chocolate samples respectively were assayed in 24fold replicates. After identification of possible outliers the OD mean and standard deviation were 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 6: Matrix-dependent and matrix-independent analytical sensitivity of the AlerTox ELISA Fish

Replicate	Sample diluent [OD]	Wine matrix [OD]	Soup matrix [OD]	Worcester sauce matrix [OD]	Asia sauce matrix [OD]	Cracker matrix [OD]	Surimi matrix [OD]	Spring roll matrix [OD]
1	0.124	0.124	0.124	0.103	0.109	0.104	0.217	0.198
2	0.112	0.145	0.140	0.096	0.097	0.100	0.178	0.196
3	0.124	0.104	0.107	0.098	0.100	0.139	0.182	0.182
4	0.135	0.127	0.132	0.096	0.106	0.126	0.198	0.190
5	0.147	0.117	0.114	0.099	0.095	0.105	0.191	0.182
6	0.150	0.114	0.104	0.096	0.088	0.099	0.185	0.178
7	0.122	0.125	0.111	0.099	0.095	0.106	0.174	0.173
8	0.139	0.110	0.117	0.089	0.090	0.100	0.186	0.179
9	0.133	0.117	0.118	0.102	0.104	0.116	0.172	0.176
10	0.116	0.135	0.121	0.108	0.112	0.101	0.182	0.178
11	0.136	0.104	0.100	0.091	0.096	0.107	0.180	0.172
12	0.140	0.112	0.116	0.100	0.096	0.114	0.239	0.180
13	0.167	0.114	0.116	0.098	0.091	0.103	0.186	0.178
14	0.143	0.107	0.109	0.098	0.086	0.095	0.172	0.172
15	0.130	0.117	0.107	0.091	0.087	0.093	0.182	0.196
16	0.122	0.112	0.115	0.093	0.109	0.097	0.177	0.191
17	0.135	0.121	0.126	0.099	0.106	0.112	0.187	0.177
18	0.135	0.118	0.097	0.092	0.107	0.103	0.174	0.192

Replicate	Sample diluent [OD]	Wine matrix [OD]	Soup matrix [OD]	Worcester sauce matrix [OD]	Asia sauce matrix [OD]	Cracker matrix [OD]	Surimi matrix [OD]	Spring roll matrix [OD]
19	0.144	0.136	0.136	0.109	0.143	0.100	0.173	0.213
20	0.141	0.116	0.106	0.102	0.096	0.110	0.187	0.182
21	0.121	0.107	0.110	0.098	0.122	0.114	0.170	0.179
22	0.121	0.103	0.100	0.095	0.090	0.113	0.205	0.184
23	0.118	0.108	0.105	0.108	0.094	0.100	0.173	0.181
24	0.118	0.128	0.142	0.100	0.101	0.100	0.177	0.192
Mean	0.132	0.118	0.116	0.098	0.101	0.107	0.185	0.184
SD	0.0130	0.0109	0.0125	0.0053	0.0126	0.0103	0.0159	0.0099
Limit of Detection	1.4 ppm	1.5 ppm	1.3 ppm	0.3 ppm	2.1 ppm	0.5 ppm	1.8 ppm	1.3 ppm

The limit of detection (LOD) is 1.4 ppm of cod. With respect to the sample matrix limits of detection vary from 0.3 to 2.1 ppm. Note that the derived limits of detection are strictly dependent on the coefficient of variation and may thus vary in every individual test. The data for sample diluent and matrices respectively were not determined in the same test runs.

The lowest positive standard (4 ppm) was defined as limit of quantification (LOQ) to assure that all uncontaminated matrices result in concentrations lower than this value.

5. Linearity

Linearity was determined by spiking wine, soup, Worcester sauce, asia sauce, cracker, surimi and spring roll samples with cod 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 7: Matrix dependent linearity of the AlerTox ELISA Fish

Red Wine

Target Value	Concentration [ppm]	Recovery [%]
100 ppm	104.4	100
50 ppm	51.2	98
25 ppm	31.0	119
12.5 ppm	13.0	100
6.25 ppm	7.0	107
	Mean [%]	105

Soup

Target Value	Concentration [ppm]	Recovery [%]
100 ppm	115.9	100

50 ppm	72.3	125
25 ppm	29.1	100
12.5 ppm	15.0	104
6.25 ppm	6.3	87
	Mean [%]	103

Worcester Sauce

Target Value	Concentration [ppm]	Recovery [%]
100 ppm	116.8	100
50 ppm	53.7	92
25 ppm	26.8	92
12.5 ppm	12.9	89
6.25 ppm	6.9	95
	Mean [%]	92

Asia Sauce

Target Value	Concentration [ppm]	Recovery [%]
100 ppm	103.3	100
50 ppm	54.2	105
25 ppm	26.3	102
12.5 ppm	10.6	82
6.25 ppm	5.3	81
	Mean [%]	93

Cracker

Target Value	Concentration [ppm]	Recovery [%]
100 ppm	102.2	100
50 ppm	55.6	109
25 ppm	24.0	94
12.5 ppm	10.3	81
6.25 ppm	4.6	72
	Mean [%]	89

Surimi

Target Value	Concentration [ppm]	Recovery [%]
100 ppm	110.2	100

50 ppm	60.1	109
25 ppm	26.5	96
12.5 ppm	10.4	75
6.25 ppm	5.5	79
	Mean [%]	90

Spring Roll

Target Value	Concentration [ppm]	Recovery [%]
100 ppm	92.4	100
50 ppm	50.2	109
25 ppm	24.6	106
12.5 ppm	10.5	91
6.25 ppm	5.5	96
	Mean [%]	100

For different matrices the mean linearity is ranging from 89% to 105%. The linearity seems to be relatively independent of the specific concentration and may moreover be affected by the intra-assay and inter-assay variation.

6. Cross-Reactivity

Since the standards are prepared from cod, the cross-reactivities of other fish species were determined relating to cod. The following cross-reactivities could be determined:

Table 8: Cross-reactivity of fish species relating to cod in the AlerTox ELISA Fish

Fish	Cross-reactivity [%]
Eel	5.7
Flounder	13.5
Perch	93.1
Trout	18.6
Pike	85.3
Herring (smoked)	1.5
Carp	79.6
Salmon	2.6
Mackerel (smoked)	0.4
Red mullet	58.9
Shark catfish	29.5
Redfish	2.8
Samlet	19.6
Sardine	7.0
Haddock	53.4

Plaice	28.9
Swordfish	0.2
Coalfish	31.0
Devilfish	1.1
Sole	2.8
Spined loach	6.3
Turbot	2.5
Tuna	0.8
Catfish	168.0
Bass	46.9
Zander	30.2

For the following foodstuffs no cross-reactivity (results < LOQ = 4 ppm) could be detected:

Table 9: Non-cross-reactive food matrices in the AlerTox ELISA Fish

Food Matrix	ppm	Food Matrix	ppm
Chicken	3.5	Potato	0
Sheep	3.0	Pork	0
Shrimp	0.6	Bean	0
Beef	0.5	Pumpkin seed	0
Macadamia	0.4	Carrot	0
Buckwheat	0.3	Cashew	0
Oat	0.3	Hazelnut	0
Mustard	0.1	Pistachio	0
Onion	0.1	Pea	0
Wheat	0	Almond	0
Barley	0	Pecan	0
Rye	0	Brazil nut	0
Soy	0	Sunflower seed	0
Egg	0	Celery	0
Corn	0	Peanut	0
Rice	0	Walnut	0
Milk	0	Millet	0
Sesame	0		

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 wine sample and a sample spiked with 50 ppm of cod 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 10: Variation of extraction temperature in the AlerTox ELISA Fish

Sample	Result 60 °C	Result 25 °C	Result 40 °C	Result 70 °C
Wine 0 ppm	0.0 ppm	0.3 ppm	0.1 ppm	0.0 ppm
Wine 50 ppm	54.6 ppm	58.1 ppm	55.4 ppm	47.6

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 11: Variation of extraction time in the AlerTox ELISA Fish

Sample	Result 15 min	Result 10 min	Result 20 min
Wine 0 ppm	0.0 ppm	0.2 ppm	0.3 ppm
Wine 50 ppm	54.5 ppm	46.5 ppm	50.2 ppm

Under consideration of the intra-assay and inter-assay variations, 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 12: Drift in the AlerTox ELISA Fish

Sample	Result 20 min	Result 16 min	Result 24 min
Wine 0 ppm	0.0 ppm	0.0 ppm	0.3 ppm
Wine 50 ppm	48.8 ppm	53.7 ppm	40.7 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.