

Validation Report

AlerTox ELISA Casein KIT3043/KT-5761

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1. Scope

The AlerTox ELISA Casein is designed for the determination of cow's milk casein (α, β, γ) 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 Casein

Replicate	Level 1	Level 2	Level 3	
1	0.47	1.78	6.16	
2	0.46	1.79	5.57	
3	0.44	1.60	6.01	
4	0.43	1.82	6.02	
5	0.48	1.80	5.43	
6	0.42	1.75	5.41	
7	0.45	1.78	4.46	
8	0.41	1.59	6.10	
9	0.47	1.80	5.19	
10	0.44	1.80	5.24	
11	0.46	1.81	6.24	
12	0.47	1.90	5.93	
13	0.48	1.70	5.21	
14	0.44	1.75	5.53	
15	0.44	1.77	5.71	
16	0.39	1.64	4.81	
17	0.39	1.82	4.82	
18	0.44	1.83	4.62	
19	0.45	1.82	4.61	
20	0.48	1.85	4.71	
Mean	0.44	1.77	5.39	
SD	0.03	0.08	0.58	RMS
CV [%]	6.1	4.6	10.7	7.6

The coefficient of variation is ranging from 4.6% to 10.7% 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 Casein

Assay No.	Level 1	Level 2	Level 3	
1	outlier	1.55	4.02	
2	0.43	1.72	4.24	
3	0.45	1.60	4.70	
4	0.39	1.86	5.48	
Mean	0.42	1.68	4.61	
SD	0.03	0.14	0.64	RMS
CV [%]	7.5	8.1	14.0	10.3

The coefficient of variation is ranging from 7.5% to 14.0% depending on the concentration.

3. Recovery

For recovery experiments different sample matrices were spiked with casein 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 Casein

Cookies

Target Value	Actual Concentration	Recovery [%]
0.6 ppm	0.69	115
2 ppm	1.86	93
6 ppm	5.51	92
	Mean	100

Soy drink

Target Value	Actual Concentration	Recovery [%]
0.6 ppm	0.61	102
2 ppm	1.95	97
6 ppm	4.95	83
	Mean	94

Sausage

Target Value	Actual Concentration	Recovery [%]
0.6 ppm	0.61	102
2 ppm	1.43	72
6 ppm	4.05	68
	Mean	80

White wine

Target Value	Actual Concentration	Recovery [%]
0.6 ppm	0.66	110
2 ppm	2.03	102
6 ppm	5.61	94
	Mean	102

Bread crumbs

Target Value	Actual Concentration	Recovery [%]
0.6 ppm	0.53	88
2 ppm	1.51	76
6 ppm	4.55	76
	Mean	80

Orange juice

Target Value	Actual Concentration	Recovery [%]
0.6 ppm	0.54	91
2 ppm	1.63	82
6 ppm	4.83	81
	Mean	84

Chocolate

Target Value	Actual Concentration	Recovery [%]
0.6 ppm	0.59	99
2 ppm	1.64	82
6 ppm	4.73	79
	Mean	86

Mean recoveries are ranging from 80% to 102% depending on the sample matrix.

In an additional validation experiment different rosé and red wines were spiked with casein to obtain various final concentrations after performing all sample pre-treatment steps. All measurements were done in 4fold replicates. The samples were measured directly after the dilution with extraction buffer, without incubation in the water bath. Tested samples and results were as follows.

Table 3a: Recovery of various rosé and red wines tested with the AlerTox ELISA Casein

Syrah, Rosé

Target Value	Actual Concentration	Recovery [%]
unspiked	0.01*	---
1.0 ppm	0.8	80
2.5 ppm	2.01	80
	Mean	80

Corbières, Red

Target Value	Actual Concentration	Recovery [%]
unspiked	0.02*	---
1.0 ppm	0.88	88
2.5 ppm	2.02	81
	Mean	85

Merlot, Red

Target Value	Actual Concentration	Recovery [%]
unspiked	0.00	---
1.0 ppm	1.01	101
2.5 ppm	2.61	104
	Mean	103

Cabernet Sauvignon, Red

Target Value	Actual Concentration	Recovery [%]
unspiked	0.03*	---
1.0 ppm	0.8	80
2.5 ppm	2.01	80
	Mean	80

* The measured value is not a real quantitative value, because it is far below LOQ and also below LOD. But it is also not a matrix effect. The value could be caused by microscopic small particles, which are floating in the wine.

Mean recoveries of the tested wines are ranging from 80% to 103% depending on the sample matrix

4. Analytical Sensitivity

For determination of the analytical sensitivity sample diluent was assayed in 21fold 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 Casein

Replicate	Sample diluent [OD]
1	0.199
2	0.184
3	0.163
4	0.163
5	0.176
6	0.188
7	0.166
8	0.207
9	0.159
10	0.154
11	0.157
12	0.261
13	0.189
14	0.170
15	0.173
16	0.164
17	0.157
18	0.157
19	0.156
20	0.157
21	0.154
Mean	0.174
SD	0.025
Limit of Detection	0.04 ppm

The calculated limit of detection is 0.04 ppm of casein and was set to 0.05 ppm.

The lowest positive standard (0.20 ppm) was defined as limit of quantification to assure that all important matrices like wheat, oats, rye, barley, cocoa, orange, soy and pork-meat result in concentrations lower than this value.

5. Linearity

Linearity was determined by spiking soy drink, sausage, white wine, orange juice, cookies, bread crumbs and chocolate samples with casein 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 Casein ELISA

Soy drink

Target Value	Concentration [ppm]	Recovery [%]
6 ppm	4.39	100
3 ppm	2.07	94
1.5 ppm	1.37	125
0.75 ppm	0.63	116
0.375 ppm	0.34	125
	Mean [%]	115

Sausage

Target Value	Concentration [ppm]	Recovery [%]
6 ppm	5.15	100
3 ppm	2.47	96
1.5 ppm	1.36	106
0.75 ppm	0.63	98
0.375 ppm	0.32	100
	Mean [%]	100

White wine

Target Value	Concentration [ppm]	Recovery [%]
6 ppm	4.60	100
3 ppm	2.79	121
1.5 ppm	1.44	126
0.75 ppm	0.83	144
0.375 ppm	0.33	116
	Mean [%]	127

Orange juice

Target Value	Concentration [ppm]	Recovery [%]
6 ppm	5.61	100
3 ppm	2.96	106
1.5 ppm	1.49	106
0.75 ppm	0.74	105
0.375 ppm	0.36	102
	Mean [%]	105

Cookies

Target Value	Concentration [ppm]	Recovery [%]
6 ppm	4.15	100
3 ppm	1.89	91
1.5 ppm	1.24	119
0.75 ppm	0.66	127
0.375 ppm	0.24	94
	Mean [%]	108

Bread crumbs

Target Value	Concentration [ppm]	Recovery [%]
6 ppm	3.38	100
3 ppm	1.70	101
1.5 ppm	1.14	134
0.75 ppm	0.55	129
0.375 ppm	0.27	130
	Mean [%]	123

Chocolate

Target Value	Concentration [ppm]	Recovery [%]
6 ppm	4.87	100
3 ppm	2.74	112
1.5 ppm	1.34	110
0.75 ppm	0.79	129
0.375 ppm	0.44	144
	Mean [%]	124

For different matrices the mean linearity is ranging from 100% to 127%. 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 Casein

Wheat	Corn	Cacao	Pork meat
Rye	Rice	Sesame	Chicken meat
Oats	Egg	Sucrose	β-Lactoglobulin
Barley	Soy	Beef meat	Orange
White wine	Rosé wine	Red wine	

The following cross-reactivity could be determined:

Food	Cross-reactivity [%]
Ewe's milk	< 1.2
Goat's milk	< 1.1

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 unspiked white wine sample and a sample spiked with 2 ppm of casein were analyzed respectively.

Variation of extraction temperature

The extraction temperature, defined as 60 °C, was changed to 40 °C and 70 °, respectively.

Table 8: Variation of extraction temperature in the AlerTox ELISA Casein

Sample	Result 60 °C	Result 40 °C	Result 70 °C
White wine 0 ppm	0 ppm	0 ppm	0 ppm
White wine 2 ppm	2.10 ppm	2.35 ppm	2.10 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 Casein

Sample	Result 15 min	Result 10 min	Result 20 min
White wine 0 ppm	0 ppm	0 ppm	0 ppm
White wine 2 ppm	2.23 ppm	2.26 ppm	2.05 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 5 minutes compared to the calibrators (20 min).

Table 10: Drift in the AlerTox ELISA Casein ELISA

Sample	Result 20 min	Result 15 min	Result 25 min
White wine 0 ppm	0 ppm	0 ppm	0.2 ppm
White wine 2 ppm	2.10 ppm	1.75 ppm	3.10 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.

Date: Mar 2018