

AlerTox[®] ELISA Egg Kit

Claim Support Summary

REF KIT3046





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1. Scope

The AlerTox® ELISA Egg Kit is based on an antibody against ovomucoid and is designed to determine egg white protein content in food.

Definitions

CV = Coefficient of variation

LOD = Limit of detection

LOQ = Limit of quantification

OD = Optical density

ppm = parts per million = mg of egg white protein per kg or L of sample (mg/kg or mg/L)

SD = Standard deviation

2. Precision

2.1 Intra-Assay Variation

The intra-assay variation (Repeatability) was determined by testing a control at 3 concentrations (1, 4 and 10 ppm) in 20-fold replicates (Table 1).

Table 1. AlerTox ELISA Egg Kit Intra-Assay Variation.

Replicate	1 ppm	4 ppm	10 ppm	
1	0.93	3.84	9.05	
2	0.97	4.09	8.62	
3	0.98	3.81	8.45	
4	0.90	3.78	8.17	
5	0.97	3.95	8.67	
6	1.06	3.81	8.92	
7	0.98	3.56	8.18	
8	0.91	3.45	8.90	
9	0.96	3.90	8.90	
10	0.96	3.90	—	
11	0.98	3.69	11.6	
12	0.96	3.77	8.98	
13	0.94	3.89	9.15	
14	0.95	3.99	8.91	
15	0.97	3.93	8.54	
16	0.95	3.87	8.72	
17	1.02	3.76	8.68	
18	0.98	3.83	9.34	
19	1.02	3.92	8.60	
20	1.06	3.76	7.80	
Mean	0.97	3.83	8.85	
SD	0.04	0.14	0.76	Mean
CV [%]	4.4	3.8	8.6	5.6

— = data not used

The coefficient of variation (CV) ranged from 3.8 to 8.6%, depending on the concentration tested.

2.2 Inter-Assay Variation

The inter-assay variation (Intermediate precision) was determined by testing controls at 3 concentrations (1, 4 and 10 ppm) in 4 different test runs of the same kit lot (Table 2).

Table 2. AlerTox ELISA Egg Kit Inter-Assay Variation.

Assay Number	1 ppm	4 ppm	10 ppm	
1	0.88	3.68	9.08	
2	0.86	3.62	8.85	
3	0.93	4.17	9.43	
4	0.99	3.79	9.08	
Mean	0.91	3.82	9.11	
SD	0.06	0.25	0.24	Mean
CV [%]	6.5	6.4	2.6	5.2

The CV ranged from 2.6 to 6.5%, depending on the concentration tested.

3. Analytical Sensitivity

To determine the analytical sensitivity of the AlerTox ELISA Egg Kit, sample diluent was assayed in 24-fold replicates. After identifying possible outliers, the mean and standard deviation of the optical density (OD) were calculated. The corresponding concentration of the mean OD + 3X standard deviation (SD) was defined as the limit of detection (LOD) (Table 3).

Table 3. Matrix-Independent Analytical Sensitivity of the AlerTox ELISA Egg Kit.

Replicate	Sample Diluent [OD]
1	0.065
2	0.052
3	—
4	0.056
5	0.049
6	0.049
7	0.053
8	0.042
9	—
10	—
11	0.047
12	0.048
13	0.046
14	0.044
15	0.044
16	0.044
17	—
18	—
19	0.055
20	0.055
21	0.047
22	0.045
23	0.043
24	0.044
Mean	0.051
SD	0.013
LOD	0.05 ppm

— = data not used

LOD is 0.05 ppm of egg white protein.

The lowest positive standard (0.4 ppm) was defined as the limit of quantification (LOQ) to ensure that measurements of important, uncontaminated matrices (e.g., milk, wheat, rye, oats, barley, cocoa and pork) result in concentrations lower than this value.



4. Recovery

For recovery experiments, different sample matrices were spiked with egg white at 3 final concentrations (1, 4 and 10 ppm) after performing all sample pre-treatment steps. The tested samples and calculated results are shown in Table 4.

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

Pasta

Spiked Value	Measured Concentration [ppm]	Recovery [%]
1 ppm	0.87	87
4 ppm	3.78	95
10 ppm	9.20	92
	Mean	91

Biscuit

Spiked Value	Measured Concentration [ppm]	Recovery [%]
1 ppm	0.85	85
4 ppm	3.71	93
10 ppm	7.14	71
	Mean	83

Sausage

Spiked Value	Measured Concentration [ppm]	Recovery [%]
1 ppm	0.95	95
4 ppm	4.02	101
10 ppm	9.87	99
	Mean	98

Cookies

Spiked Value	Measured Concentration [ppm]	Recovery [%]
1 ppm	0.83	83
4 ppm	3.58	90
10 ppm	8.23	82
	Mean	85

Dark Chocolate

Spiked Value	Measured Concentration [ppm]	Recovery [%]
1 ppm	0.78	78
4 ppm	3.64	91
10 ppm	7.72	77
	Mean	82

Mean recoveries ranged from 82 to 98%, depending on the sample matrix.

5. Linearity

Linearity was determined by spiking pasta, biscuit, cookies, chocolate and sausage samples with egg white and testing serial dilutions (10 to 0.625 ppm) of the resulting extracts (Table 5). For calculating linearity, the highest concentration was defined as the reference value (100%), and further dilutions were expressed as a percentage of this reference after accounting for the dilution factor.

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

Pasta

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	9.73	Reference* (100)
5 ppm	5.30	109
2.5 ppm	2.76	113
1.25 ppm	1.25	103
0.625 ppm	0.57	94
	Mean	105

Biscuit

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	9.89	Reference* (100)
5 ppm	5.31	107
2.5 ppm	2.92	118
1.25 ppm	1.50	121
0.625 ppm	0.62	100
	Mean	112

Sausage

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	10.35	Reference* (100)
5 ppm	5.18	100
2.5 ppm	2.79	108
1.25 ppm	1.14	88
0.625 ppm	0.40	62
	Mean	90

Cookies

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	9.63	Reference* (100)
5 ppm	4.65	97
2.5 ppm	2.66	110
1.25 ppm	1.32	110
0.625 ppm	0.75	125
	Mean	110

* The highest concentration was defined as the reference value (100%). Further dilutions were expressed as a percentage of this reference after accounting for the dilution factor.

**Dark Chocolate**

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	8.60	Reference* (100)
5 ppm	4.09	95
2.5 ppm	2.43	113
1.25 ppm	0.96	89
0.625 ppm	0.41	76
	Mean	93

* The highest concentration was defined as the reference value (100%). Further dilutions were expressed as a percentage of this reference after accounting for the dilution factor.

For different matrices, the mean linearity ranged from 90 to 112%. The linearity was independent of the specific concentration and may have been affected by the precision, as stated in Section 2.

6. Cross-Reactivity

No cross-reactivity is defined as a result that is less than the LOQ. For the AlerTox ELISA Egg Kit, the LOQ is 0.4 ppm egg white protein. During testing, no cross-reactivity was detected in the foods included in Table 6.

Table 6. Non-Cross-Reactive Food Matrices with the AlerTox ELISA Egg Kit.

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

* Chicken (cooked), duck (raw), thyme and turkey (raw) showed results between 0.5 LOQ and 1 LOQ and may provide values above the LOQ.



The following cross-reactivities were determined (Table 7):

Table 7. Cross-Reactive Food Matrices with the AlerTox ELISA Egg Kit.

Cross-Reactive Matrix	Percent Cross-Reactivity (%)
Conalbumin	2.6
Ovalbumin	0.25
Lysozyme	< 0.0003
Chicken (raw)	0.00006

7. Robustness

Robustness was determined by the variation of different 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 cookie sample and a sample spiked with 4 ppm of egg white 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).

Table 8. Variation of Extraction Temperature with the AlerTox ELISA Egg Kit.

Sample	Result 60 °C	Result 25 °C	Result 40 °C	Result 70 °C
Cookie, 0 ppm egg white	0 ppm	0 ppm	0 ppm	0 ppm
Cookie, 4 ppm egg white	4.41 ppm	2.58 ppm	2.60 ppm	3.99 ppm

The results differ significantly. For accurate results, the 60 °C extraction temperature specified in the instruction manual must be used.

7.2 Variation of Extraction Time

The extraction time, defined as 15 min, was changed to 10 min and 20 min (Table 9).

Table 9. Variation of Extraction Time with the AlerTox ELISA Egg Kit.

Sample	Result 15 min	Result 10 min	Result 20 min
Cookie, 0 ppm egg white	0 ppm	0 ppm	0 ppm
Cookie, 4 ppm egg white	4.41 ppm	4.44 ppm	3.66 ppm

Considering the precision data (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 4 minutes compared to the calibrators (20 min) (Table 10).

Table 10. Drift in the AlerTox ELISA Soy Kit.

Sample	Result 20 min	Result 16 min	Result 24 min
Cookie, 0 ppm egg white	0 ppm	0 ppm	0 ppm
Cookie, 4 ppm egg white	4.41 ppm	2.70 ppm	4.32 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.

8. Summary

- The AlerTox ELISA Egg Kit has an LOD of 0.05 ppm (mg of egg white protein per kg or L of sample) and an LOQ of 0.4 ppm of egg white protein (mg/kg or mg/L). (See the Appendix for the LOD and LOQ for wine samples.)
- The AlerTox ELISA Egg Kit provides consistent, precise results, as indicated by results of the intra- and inter-assay studies (CV < 10%, Section 2).
- Robust recovery (Section 4) and linearity (Section 5) mean that samples and their dilutions are quantitated accurately.
- Extensive cross-reactivity studies showed high specificity (Section 6, Table 6), except for low cross-reactivity with conalbumin, ovalbumin, lysozyme and raw chicken (Section 6, Table 7).
- **Important tips:**
 - As specified in the instructions, a 60 °C extraction temperature must be used for accurate results.
 - Drift in large test runs can be avoided by pipetting calibrators once before and once after the samples and using the calibrator mean values for the calculations.

Appendix: Wine Matrices

A1. Scope

This appendix presents results from validation studies for the AlerTox ELISA Egg Kit using white and red wine matrices.

A2. Recovery of Egg White from Wine Samples

For recovery experiments, wine samples were spiked with egg white at 2 final concentrations (1 and 4 ppm) after performing all sample pre-treatment steps. The tested samples and calculated results are shown in Table A1.

Table A1. Recovery of Wine Samples Tested with the AlerTox ELISA Egg Kit.

Matrix	Spiked Value (Egg White)	Measured Concentration [ppm]	Recovery [%]
White wine	1 ppm	1.02	110
	4 ppm	4.68	
Red wine	1 ppm	1.01	104
	4 ppm	4.23	

Recovery rates were 104% and 110% for red and white wine, respectively. Considering the intra- and inter-assay variation (Section 2), the results did *not* significantly differ from 100%.

A2. LOD and LOQ for Wine Samples

To determine the LOD and LOQ, wine samples were assayed in 24-fold replicates. After identifying possible outliers, the mean and standard deviation of the optical density (OD) were calculated. The corresponding concentration of the mean OD + 3X standard deviation (SD) was defined as the LOD (Table A2). The corresponding concentration of the mean OD + 9X SD was defined as the LOQ (Table A2).

Table A2. LOD and LOQ of the AlerTox ELISA Egg Kit with Wine Samples.

Replicate	White Wine [OD]	Red Wine [OD]
1	0.017	0.017
2	0.016	0.018
3	0.010	0.012
4	0.015	0.018
5	0.017	0.017
6	0.013	—
7	0.019	0.020
8	0.014	0.015
9	0.015	0.019
10	0.013	0.016
11	0.008	0.013
12	0.013	0.017
13	0.012	0.017
14	0.011	0.014
15	0.017	0.022
16	0.011	0.016
17	0.014	0.020
18	0.025	0.018
19	0.009	0.014
20	0.014	0.017
21	0.011	0.016
22	0.009	0.015
23	0.017	0.022
24	0.014	0.017
Mean	0.014	0.017
SD	0.004	0.003
LOD	0.02 ppm	0.02 ppm
LOQ	0.06 ppm	0.05 ppm

— = data not used

The LOD is 0.02 ppm of egg white protein in white and red wine samples.

The LOQ is 0.06 ppm of egg white protein in white wine and 0.05 ppm of egg white protein in red wine.