AlerTox® ELISA Ovalbumin Kit

Claim Support Summary

REF KIT3045

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1. Scope

The AlerTox® ELISA Ovalbumin Kit is designed to determine ovalbumin content in wine and other food. This report describes the validation process and its results.

Definitions

CV = Coefficient of variation

IQR = Interquartile range

LOD = Limit of detection

LOQ = Limit of quantification

OD = Optical density

ppb = parts per billion = µg of ovalbumin per kg or L of sample (µg/kg or µg/L)

PT = Proficiency testing

SD = Standard deviation

2. Precision

2.1 Intra-Assay Variation

The intra-assay variation (repeatability) was determined by testing a control at 3 concentrations (50, 150 and 300 ppb) in 20-fold replicates (Table 1).

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

Replicate	50 ppb	150 ppb	300 ppb	
1	51.4	188	356	
2	52.1	176	350	
3	49.5	175	347	
4	50.0	176	360	
5	51.0	176	357	
6	48.7	168	349	
7	50.2	177	357	
8	45.6	160	322	
9	51.9	177	343	
10	49.8	175	347	
11	49.8	171	344	
12	50.9	171	355	
13	50.0	176	362	
14	50.7	167	349	
15	49.1	174	353	
16	47.9	158	327	
17	53.0	172	354	
18	52.6	171	346	
19	51.0	171	339	
20	52.6	174	349	
Mean	50.4	173	348	
SD	1.77	6.44	10.1	Mean
CV [%]	**3.5**	**3.7**	**2.9**	**3.4**

The coefficient of variation (CV) ranged from 2.9 to 3.7%, depending on the concentration tested.

2.2 Inter-Assay Variation

The inter-assay variation (intermediate precision) was determined by testing a control at 3 concentrations (50, 150 and 300 ppb) in 4 different test runs of the same kit lot (Table 2).

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

Assay Number	50 ppb	150 ppb	300 ppb	
1	51.7	170	317	
2	49.8	164	321	
3	50.7	168	327	
4	47.8	170	305	
Mean	50.0	168	318	
SD	1.63	2.71	9.69	Mean
CV [%]	**3.3**	**1.6**	**3.1**	**2.7**

The CV ranged from 1.6 to 3.3%, depending on the concentration tested.

3. Analytical Sensitivity

To determine the analytical sensitivity of the AlerTox ELISA Ovalbumin Kit, sample diluent (matrix independent) and ovalbumin-free wine samples (matrix dependent) were assayed in 24-fold replicates. The data from sample diluent and matrices were assessed in the same test runs.

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 and Matrix-Dependent Analytical Sensitivity of the AlerTox ELISA Ovalbumin Kit.

Replicate	Sample Diluent [OD]	White Wine Matrix [OD]	Red Wine Matrix [OD]
1	0.069	0.017	0.024
2	0.061	0.019	0.023
3	0.063	0.025	0.023
4	0.068	0.024	0.033
5	0.071	0.029	0.033
6	0.064	0.030	0.040
7	0.066	0.030	0.028
8	0.061	0.041	0.018
9	0.067	0.024	0.024
10	0.061	0.024	0.025
11	0.069	0.024	0.024
12	0.064	0.023	0.026
13	0.066	0.026	0.016
14	0.066	0.040	0.028
15	0.062	0.027	0.036
16	0.058	0.026	0.019
17	0.055	0.017	0.024
18	0.054	0.025	0.029
19	0.064	0.029	0.038
20	0.075	0.030	0.034
21	0.053	0.042	0.029
22	0.069	0.029	0.028
23	0.064	0.027	0.037
24	0.075	0.033	0.017
Mean	0.064	0.028	0.027
SD	0.006	0.007	0.007
LOD [ppb]	**4.4 ppb**	**< 0.05 ppb**	**< 0.05 ppb**

— = data not used.

LODs vary from < 0.05 to 4.4 ppb, depending on the matrix. Note that the derived LODs are strictly dependent on the CV and thus may vary in individual tests.

The lowest positive standard (25 ppb) was defined as the limit of quantification (LOQ) to ensure that measurements of all uncontaminated matrices result in concentrations lower than this value.

4. Recovery

For recovery experiments with wine, different sample matrices were spiked with ovalbumin at various final concentrations (50 to 300 ppb) after performing all sample pre-treatment steps. The tested samples and calculated results are shown in Table 4.

Table 4. Recovery of Wine Samples Tested with the AlerTox ELISA Ovalbumin Kit.

Sample	Spiked Value	Measured Concentration [ppb]	Recovery [%]
White wine 1	50 ppb	54.3	109
	150 ppb	172	115
	300 ppb	318	106
White wine 2	100 ppb	103	103
Red wine 1	50 ppb	54.5	109
	150 ppb	163	109
	300 ppb	291	97
Red wine 2	100 ppb	95.2	95
Rosé wine	100 ppb	104	104
		Mean	105

The mean recovery for wine is 105%.

5. Linearity

Linearity was determined by spiking wine samples with ovalbumin and testing serial dilutions (400 to 25 ppb) of the resulting matrices (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 Ovalbumin Kit for Wine Samples.

White Wine

Spiked Value	Measured Concentration [ppb]	Recovery [%]
400 ppb	386	Reference* (100)
200 ppb	185	96
100 ppb	90.8	94
50 ppb	44.9	93
25 ppb	20.7	86
	Mean	94

* 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.

Red Wine

Spiked Value	Measured Concentration [ppb]	Recovery [%]
400 ppb	400	Reference* (100)
200 ppb	209	104
100 ppb	99.2	99
50 ppb	46.6	93
25 ppb	20.4	82
	Mean	96

* 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 wine matrices, the mean linearity ranged from 94 to 95%. 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 Ovalbumin Kit, the LOQ is 25 ppb of ovalbumin. 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 Ovalbumin 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
Chicken	Chickpea	Chili	Cinnamon	Clove
Cocoa	Coconut	Cod	Corn	Cumin
Dill	Duck	Fennel	Fenugreek	Flaxseed
Garden cress	Garlic (fresh)	Garlic (granulated)	Gelatin, cow	Gelatin, fish*
Ginger (ground)	Ginger (fresh)	Gliadin	Guar gum	Gum arabic*
Hazelnut	Horseradish	Isinglass	Kidney bean	Kiwi
Lamb	Leek	Lentil	Lupine	Macadamia
Milk, cow	Milk, cow (non-fat dry)	Milk, goat	Milk, sheep	Mustard, yellow
Nutmeg	Oats	Onion	Paprika	Pea
Peach	Peanut	Pecan	Pepper, black	Pine seed
Pistachio	Poppy	Pork	Potato	Prawn (cooked)
Prawn (raw)	Pumpkin seed	Radish	Rapeseed	Rice
Rye	Saccharose	Sesame	Shrimps	Soy lecithin
Split pea*	Sunflower seed	Thyme	Tofu	Tomato
Turkey*	Turmeric	Walnut	Wheat	

* Gelatin (fish), split pea, turkey and gum arabic 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 Ovalbumin Kit.

Cross-Reactive Matrix	Percent Cross-Reactivity (%)
Whole egg powder	15
Conalbumin*	< 0.2
Lysozyme*	< 0.02
Ovomucoid*	< 0.02

* Since the tested egg proteins may not be pure, it is possible that the minor cross-reactivities of conalbumin, lysozyme and ovomucoid are from lysozyme contamination in the tested material.

7. Robustness: Drift

Robustness was determined by varying the sample incubation time defined in the instruction manual. The results obtained under various conditions (incubation extended or reduced by 5 minutes) were compared with those obtained by following the instruction manual (20-minute incubation). An unspiked red wine sample and a sample spiked with 100 ppb ovalbumin were analyzed (Table 10).

Table 10. Drift in the AlerTox ELISA Ovalbumin Kit.

Sample	Result, 20 min	Result, 15 min	Result, 25 min
Red wine, 0 ppb ovalbumin	0 ppb	0 ppb	0 ppb
Red wine, 100 ppb ovalbumin	**92.1 ppb**	101 ppb	66.6 ppb

The results differ 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 Ovalbumin Kit is sensitive with a matrix-independent LOD of 4 ppb (µg of ovalbumin per kg or L of sample) and a matrix-dependent LOD of < 0.05 ppb (µg/kg or µg/L). The LOQ is 25 ppb ovalbumin (µg/kg or µg/L).
- The AlerTox ELISA Ovalbumin 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) with potential cross-reactivity with conalbumin, lysozyme and ovomucoid.
- **Important**: 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.