AlerTox® ELISA Lysozyme Kit

Claim Support Summary

REF KIT3044

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1. Scope

The AlerTox® ELISA Lysozyme Kit is designed to determine lysozyme content in 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 lysozyme 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, 100 and 200 ppb) in 20-fold replicates (Table 1).

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

Replicate	50 ppb	100 ppb	200 ppb	
1	56.9	108	250	
2	53.6	108	224	
3	52.8	104	229	
4	54.8	110	240	
5	54.4	108	244	
6	51.4	102	216	
7	61.6	103	234	
8	48.0	108	210	
9	54.0	106	251	
10	53.0	106	228	
11	53.7	110	223	
12	55.1	110	238	
13	55.3	105	226	
14	53.5	106	218	
15	52.5	103	231	
16	52.7	105	198	
17	54.8	107	225	
18	53.5	108	224	
19	55.3	105	210	
20	55.2	104	221	
Mean	54.1	106	227	
SD	2.5	2.5	13.6	Mean
CV [%]	**4.7**	**2.3**	**6.0**	**4.3**

The coefficient of variation (CV) ranged from 2.3 to 6.0%, 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, 100 and 200 ppb) in 4 different test runs of the same kit lot (Table 2).

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

Assay Number	50 ppb	100 ppb	200 ppb	
1	49.9	98.2	184	
2	50.4	101	181	
3	51.8	103	192	
4	49.7	103	184	
Mean	50.5	101	185	
SD	0.9	2.3	4.9	Mean
CV [%]	**1.8**	**2.3**	**2.6**	**2.2**

The CV ranged from 1.8 to 2.6%, depending on the concentration tested.

3. Analytical Sensitivity

To determine the analytical sensitivity of the AlerTox ELISA Lysozyme Kit, sample diluent (matrix independent) and various representative, lysozyme-free wine and cheese samples (matrix dependent) were assayed in 24-fold replicates. The data from sample diluent and matrices were assessed in separate 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 Lysozyme Kit.

Replicate	Sample Diluent [OD]	White Wine Matrix [OD]	Red Wine Matrix [OD]	Camembert Matrix [OD]	Maasdamer Matrix [OD]	Soft Cheese Matrix [OD]
1	0.056	0.040	0.048	0.005	0.007	0.006
2	0.040	0.041	0.045	0.003	0.004	0.004
3	0.064	0.046	0.038	0.004	0.004	0.003
4	0.042	0.040	0.045	0.006	0.007	0.004
5	0.038	0.056	0.045	0.007	0.006	0.006
6	0.038	0.047	0.044	0.004	0.005	0.004
7	0.040	0.047	0.047	0.006	0.007	0.005
8	0.042	0.043	0.041	0.009	0.007	0.005
9	0.050	0.043	0.040	0.006	0.005	0.004
10	0.043	0.046	0.049	0.004	0.003	0.003
11	0.043	0.044	0.048	0.003	0.003	0.004
12	0.042	0.048	0.044	0.006	0.004	0.004
13	0.048	0.063	0.048	0.006	0.005	0.004
14	0.040	0.048	0.043	0.006	0.008	0.004
15	0.040	0.053	0.043	0.009	0.003	0.004
16	0.042	0.053	0.047	0.005	0.005	0.007
17	0.052	0.057	0.053	0.004	0.006	0.005
18	0.048	0.051	0.047	—	0.004	0.003
19	0.044	0.046	0.043	0.008	0.003	0.003
20	0.042	0.044	0.040	0.007	0.004	0.005
21	0.056	0.046	0.048	0.005	0.005	0.005
22	0.039	0.043	0.042	0.007	0.004	0.005
23	0.040	0.049	0.046	0.009	0.005	0.004
24	0.050	0.045	0.041	0.009	0.007	0.007
Mean	**0.045**	**0.047**	**0.045**	**0.006**	**0.005**	**0.005**
SD	**0.0067**	**0.00578**	**0.00358**	**0.00191**	**0.00152**	**0.00114**
LOD [ppb]	**2.3 ppb**	**2.2 ppb**	**1.2 ppb**	**3.0 ppb**	**2.5 ppb**	**2.0 ppb**

— = data not used.

LODs vary from 1.2 to 3.0 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

4.1 Wine

For recovery experiments with wine, different sample matrices were spiked with lysozyme at various final concentrations (25 to 200 ppb) after performing all sample pre-treatment steps. The tested samples (unspiked and spiked) and calculated results are shown in Table 4.

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

Sample	Spiked Value	Measured Concentration [ppb]	Recovery [%]
White wine 1	0 ppb	0.4	—
	25 ppb	22.3	89
	50 ppb	62.1	124
	100 ppb	117	117
	200 ppb	228	114
White wine 2	0 ppb	0.0	—
	100 ppb	86.0	86
Red wine 1	0 ppb	0.0	—
	25 ppb	23.5	94
	50 ppb	44.2	88
	100 ppb	96.2	96
	200 ppb	185	92
Red wine 2	0 ppb	0.0	—
	100 ppb	86.5	87
Rosé wine	0 ppb	0.0	—
	25 ppb	23.3	93
	100 ppb	90.3	90
		Mean	98

The mean recovery for wine is 98%.

4.2 Cheese

For recovery experiments with cheese, different sample matrices were spiked with lysozyme at 4 final concentrations (125, 250, 500 and 1,000 ppb) after performing all sample pre-treatment steps. The tested samples (unspiked and spiked) and calculated results are shown in Table 5.

Table 5. Recovery of Cheese Samples Tested with the AlerTox ELISA Lysozyme Kit.

Sample	Spiked Value	Measured Concentration [ppb]	Recovery [%]
Camembert 35% fat in dry weight	0 ppb	0.0	—
	125 ppb	108	86
	250 ppb	195	78
	500 ppb	363	73
	1,000 ppb	803	80
Maasdamer 45% fat in dry weight	0 ppb	0.0	—
	125 ppb	110	88
	250 ppb	253	101
	500 ppb	451	90
	1,000 ppb	1,065	107
Soft cheese 50% fat in dry weight	0 ppb	0.0	—
	125 ppb	115	92
	250 ppb	214	86
	500 ppb	378	76
	1,000 ppb	802	80
		Mean	87

The mean recovery for cheese is 87%.

5. Linearity

5.1 Wine

Linearity was determined by spiking wine samples with lysozyme and testing serial dilutions (250 to 15.625 ppb) of the resulting matrices (Table 6). For calculating linearity, the highest concentration was defined as the reference value (100%), and subsequent dilutions were expressed as a percentage of this reference after accounting for the dilution factor.

Table 6. Matrix-Dependent Linearity of the AlerTox ELISA Lysozyme Kit for Wine Samples.

White Wine

Spiked Value	Measured Concentration [ppb]	Recovery [%]
250 ppb	249	Reference* (100)
125 ppb	120	96
62.5 ppb	53.5	86
31.25 ppb	23.7	76
15.625 ppb	17.9	115
	Mean	95

* 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 [%]
250 ppb	239	Reference* (100)
125 ppb	111	93
62.5 ppb	50.5	84
31.25 ppb	23.5	79
15.625 ppb	11.8	79
	Mean	87

* 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 87 to 95%. The linearity may have been affected by the precision, as stated in Section 2.

5.2 Cheese

Linearity was determined by spiking cheese samples with lysozyme and testing serial dilutions (1,000 to 62.5 ppb) of the resulting matrices (Table 7). For calculating linearity, the highest concentration was defined as the reference value (100%), and subsequent dilutions were expressed as a percentage of this reference after accounting for the dilution factor.

Table 7. Matrix-Dependent Linearity of the AlerTox ELISA Lysozyme Kit for Cheese Samples.

Camembert, 35% fat in dry weight

Spiked Value	Measured Concentration [ppb]	Recovery [%]
1,000 ppb	803	Reference* (100)
500 ppb	389	97
250 ppb	196	98
125 ppb	100	99
62.5 ppb	48	96
	Mean	97

Maasdamer, 45% fat in dry weight

Spiked Value	Measured Concentration [ppb]	Recovery [%]
1,000 ppb	1,065	Reference* (100)
500 ppb	533	100
250 ppb	270	101
125 ppb	143	107
62.5 ppb	55	83
	Mean	98

* 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.

Soft Cheese, 50% fat in dry weight

Spiked Value	Measured Concentration [ppb]	Recovery [%]
1,000 ppb	802	Reference* (100)
500 ppb	384	96
250 ppb	189	94
125 ppb	93	93
62.5 ppb	42	84
	Mean	92

* 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 cheese matrices, the mean linearity ranged from 92 to 98%. The linearity 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 Lysozyme Kit, the LOQ is 25 ppb of lysozyme. During testing, no cross-reactivity was detected in the foods included in Table 8.

Table 8. Non-Cross-Reactive Food Matrices with the AlerTox ELISA Lysozyme Kit.

Non-Cross-Reactive Matrices				
Adzuki bean	Almond	Apricot	Barley	Bean, white
Beef (raw)	Beef (cooked)	Brazil nut	Buckwheat	Cabbage, white
Caraway seeds	Cardamom	Carob gum	Carrot	Cashew
Cayenne	Celery	Cherry	Chestnut	Chia seeds
Chicken	Chickpea	Chili	Cinnamon	Clove
Cocoa	Coconut	Cod	Corn	Cumin
Dill	Duck	Fennel	Fenugreek	Flaxseed
Garden cress	Garlic (fresh)	Garlic (granulated)	Gelatin, cow	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	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 flour	Soy lecithin	Split pea	Sunflower seed
Thyme	Tofu	Tomato	Turkey	Turmeric
Walnut			Wheat	

* Goat's milk showed results between 0.5 LOQ and 1 LOQ and may provide values above the LOQ.

The following cross-reactivities were determined (Table 9):

Table 9. Cross-Reactive Food Matrices with the AlerTox ELISA Lysozyme Kit.

Cross-Reactive Matrix	Percent Cross-Reactivity (%)
Egg white protein, total	2.2
Conalbumin*	< 0.0001
Ovalbumin*	< 0.0001
Ovomucoid*	< 0.0001

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

7. Robustness: Drift

Robustness was determined by the variation of 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 red wine sample and a sample spiked with 100 ppb lysozyme were analyzed.

In contrast to the test procedure 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).

Table 10. Drift in the AlerTox ELISA Lysozyme Kit.

Sample	Result, 20 min	Result, 15 min	Result, 25 min
Red wine, 0 ppb lysozyme	0 ppb	0 ppb	0 ppb
Red wine, 100 ppb lysozyme	**90.9 ppb**	75.4 ppb	99.8 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 Lysozyme Kit is sensitive with a matrix-independent LOD of 2.3 ppb (µg of lysozyme per kg or L of sample) and an LOD range of 1.2 to 3.0 ppb (µg/kg or µg/L), depending on the matrix. The LOQ is 25 ppb lysozyme (µg/kg or µg/L).
- The AlerTox ELISA Lysozyme 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 most samples and their dilutions are quantitated accurately.
- Extensive cross-reactivity studies showed high specificity (Section 6) with potential cross-reactivity with conalbumin, ovalbumin 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.