

# AlerTox® ELISA Pistachio KIT3054

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# AlerTox® ELISA Pistachio | KIT3054

## 1. Scope

The AlerTox® ELISA Pistachio is designed for the determination of pistachio 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 Pistachio**

| Replicate     | Level 1    | Level 2    | Level 3    |            |
|---------------|------------|------------|------------|------------|
| 1             | 4.8        | 8.8        | 37.3       |            |
| 2             | 3.7        | 8.6        | 34.7       |            |
| 3             | 4.1        | 8.9        | 32.5       |            |
| 4             | 3.6        | 8.2        | 35.6       |            |
| 5             | 3.9        | 7.3        | 38.7       |            |
| 6             | 4.0        | 7.9        | 34.1       |            |
| 7             | 4.0        | 9.3        | 35.0       |            |
| 8             | 4.2        | 8.3        | 37.2       |            |
| 9             | 3.6        | 8.9        | 35.4       |            |
| 10            | 4.1        | 9.3        | 35.2       |            |
| 11            | 3.8        | 9.2        | 35.7       |            |
| 12            | 3.5        | 8.6        | 37.4       |            |
| 13            | 4.0        | 8.2        | 41.3       |            |
| 14            | 4.1        | 9.1        | 35.9       |            |
| 15            | 3.9        | 7.5        | 40.5       |            |
| 16            | 4.0        | 8.5        | 39.6       |            |
| 17            | 3.5        | 7.5        | 37.5       |            |
| 18            | 4.6        | 8.1        | 35.0       |            |
| 19            | 4.2        | 8.0        | 36.3       |            |
| 20            | 4.0        | 7.1        | 36.9       |            |
| <b>Mean</b>   | 4.0        | 8.4        | 36.6       |            |
| <b>SD</b>     | 0.3        | 0.7        | 2.2        | <b>RMS</b> |
| <b>CV [%]</b> | <b>8.1</b> | <b>8.0</b> | <b>6.0</b> | <b>7.4</b> |

The coefficient of variation is ranging from 6.0% to 8.1% depending on the concentration.

RMS = Root Mean Square

## 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 Pistachio**

| Assay No.     | Level 1    | Level 2     | Level 3     |            |
|---------------|------------|-------------|-------------|------------|
| 1             | 4.5        | 9.0         | 32.7        |            |
| 2             | 4.3        | 10.0        | 38.7        |            |
| 3             | 4.5        | 11.0        | 41.8        |            |
| 4             | 4.0        | 8.4         | 36.1        |            |
| <b>Mean</b>   | 4.3        | 9.6         | 37.3        |            |
| <b>SD</b>     | 0.2        | 1.1         | 3.8         | <b>RMS</b> |
| <b>CV [%]</b> | <b>5.2</b> | <b>11.9</b> | <b>10.3</b> | <b>9.1</b> |

The coefficient of variation is ranging from 5.2% to 11.9% depending on the concentration.

## 3. Recovery

For recovery experiments, different sample matrices were spiked with pistachio 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 Pistachio**

### Cookies

| Target Value | Actual Concentration | Recovery [%] |
|--------------|----------------------|--------------|
| 4 ppm        | 3.4                  | 85           |
| 10 ppm       | 10.2                 | 102          |
| 40 ppm       | 37.8                 | 94           |
|              | <b>Mean</b>          | <b>94</b>    |

### Cornflakes

| Target Value | Actual Concentration | Recovery [%] |
|--------------|----------------------|--------------|
| 4 ppm        | 3.6                  | 89           |
| 10 ppm       | 10.0                 | 100          |
| 40 ppm       | 38.9                 | 97           |
|              | <b>Mean</b>          | <b>96</b>    |

### Ice Cream

| Target Value | Actual Concentration | Recovery [%] |
|--------------|----------------------|--------------|
| 4 ppm        | 4.0                  | 100          |
| 10 ppm       | 8.2                  | 82           |
| 40 ppm       | 34.1                 | 85           |
|              | <b>Mean</b>          | <b>89</b>    |

### Chocolate

| Target Value | Actual Concentration | Recovery [%] |
|--------------|----------------------|--------------|
| 4 ppm        | 3.1                  | 78           |
| 10 ppm       | 8.4                  | 84           |
| 40 ppm       | 32.5                 | 81           |
|              | <b>Mean</b>          | <b>81</b>    |

### Sausage

| Target Value | Actual Concentration | Recovery [%] |
|--------------|----------------------|--------------|
| 4 ppm        | 3.6                  | 89           |
| 10 ppm       | 9.3                  | 93           |
| 40 ppm       | 35.1                 | 88           |
|              | <b>Mean</b>          | <b>90</b>    |

Mean recoveries are ranging from 81% to 96% depending on the sample matrix.

## 4. Analytical Sensitivity

For determination of the analytical sensitivity, sample diluent and pistachio free cookies, cornflakes, ice-cream, chocolate and sausage 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 4: Matrix-dependent and matrix-independent analytical sensitivity of the AlerTox® ELISA Pistachio**

| Replicate                 | Sample diluent [OD] | Cookie matrix [OD] | Cornflakes matrix [OD] | Ice Cream matrix [OD] | Chocolate matrix [OD] | Sausage matrix [OD] |
|---------------------------|---------------------|--------------------|------------------------|-----------------------|-----------------------|---------------------|
| 1                         | 0.041               | 0.034              | 0.039                  | 0.052                 | 0.033                 | 0.021               |
| 2                         | 0.048               | 0.035              | 0.034                  | 0.047                 | 0.028                 | 0.021               |
| 3                         | 0.051               | 0.030              | 0.029                  | 0.043                 | 0.030                 | 0.019               |
| 4                         | 0.045               | 0.045              | 0.040                  | 0.068                 | 0.028                 | 0.020               |
| 5                         | 0.047               | 0.035              | 0.037                  | 0.047                 | 0.033                 | 0.023               |
| 6                         | 0.037               | 0.035              | 0.034                  | 0.042                 | 0.028                 | 0.021               |
| 7                         | 0.039               | 0.035              | 0.037                  | 0.041                 | 0.029                 | 0.022               |
| 8                         | 0.064               | 0.037              | 0.031                  | 0.043                 | 0.032                 | 0.024               |
| 9                         | 0.040               | 0.039              | 0.038                  | 0.049                 | 0.034                 | 0.032               |
| 10                        | 0.039               | 0.040              | 0.036                  | 0.044                 | 0.035                 | 0.020               |
| 11                        | 0.040               | 0.034              | 0.028                  | 0.037                 | 0.028                 | 0.018               |
| 12                        | 0.049               | 0.071              | 0.039                  | 0.059                 | 0.054                 | 0.021               |
| 13                        | 0.038               | 0.041              | 0.038                  | 0.045                 | 0.033                 | 0.026               |
| 14                        | 0.038               | 0.045              | 0.035                  | 0.040                 | 0.029                 | 0.022               |
| 15                        | 0.040               | 0.038              | 0.038                  | 0.043                 | 0.032                 | 0.023               |
| 16                        | 0.039               | 0.034              | 0.030                  | 0.041                 | 0.028                 | 0.026               |
| 17                        | 0.037               | 0.039              | 0.036                  | 0.048                 | 0.033                 | 0.021               |
| 18                        | 0.046               | 0.034              | 0.034                  | 0.039                 | 0.031                 | 0.018               |
| 19                        | 0.049               | 0.032              | 0.030                  | 0.033                 | 0.030                 | 0.018               |
| 20                        | 0.047               | 0.055              | 0.039                  | 0.057                 | 0.048                 | 0.020               |
| 21                        | 0.037               | 0.035              | 0.040                  | 0.045                 | 0.035                 | 0.024               |
| 22                        | 0.037               | 0.037              | 0.031                  | 0.044                 | 0.030                 | 0.023               |
| 23                        | 0.045               | 0.033              | 0.033                  | 0.046                 | 0.032                 | 0.026               |
| 24                        | 0.050               | 0.034              | 0.037                  | 0.036                 | 0.035                 | 0.027               |
| <b>Mean</b>               | <b>0.043</b>        | <b>0.039</b>       | <b>0.035</b>           | <b>0.045</b>          | <b>0.033</b>          | <b>0.022</b>        |
| <b>SD</b>                 | <b>0.006</b>        | <b>0.009</b>       | <b>0.004</b>           | <b>0.008</b>          | <b>0.006</b>          | <b>0.003</b>        |
| <b>Limit of Detection</b> | <b>0.13 ppm</b>     | <b>0.18 ppm</b>    | <b>0.01 ppm</b>        | <b>0.20 ppm</b>       | <b>0.09 ppm</b>       | <b>0.17 ppm</b>     |

The limit of detection (LOD) is 0.13 ppm of pistachio. With respect to the sample matrix limits of detection vary from 0.01 to 0.20 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 (1 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 cookie, cornflakes, ice-cream, chocolate and sausage samples with pistachio 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 Pistachio**

### Cookies

| Target Value | Concentration [ppm] | Recovery [%] |
|--------------|---------------------|--------------|
| 40 ppm       | 37.7                | 100          |
| 20 ppm       | 20.2                | 107          |
| 10 ppm       | 8.2                 | 87           |
| 5 ppm        | 4.8                 | 101          |
| 2.5 ppm      | 2.4                 | 100          |
|              | <b>Mean [%]</b>     | <b>99</b>    |

### Cornflakes

| Target Value | Concentration [ppm] | Recovery [%] |
|--------------|---------------------|--------------|
| 40 ppm       | 37.9                | 100          |
| 20 ppm       | 19.9                | 105          |
| 10 ppm       | 7.8                 | 83           |
| 5 ppm        | 3.9                 | 81           |
| 2.5 ppm      | 2.1                 | 90           |
|              | <b>Mean [%]</b>     | <b>90</b>    |

### Ice-cream

| Target Value | Concentration [ppm] | Recovery [%] |
|--------------|---------------------|--------------|
| 40 ppm       | 37.6                | 100          |
| 20 ppm       | 23.5                | 125          |
| 10 ppm       | 10.1                | 107          |
| 5 ppm        | 4.4                 | 93           |
| 2.5 ppm      | 2.6                 | 111          |
|              | <b>Mean [%]</b>     | <b>109</b>   |

## Chocolate

| Target Value    | Concentration [ppm] | Recovery [%] |
|-----------------|---------------------|--------------|
| 40 ppm          | 37.0                | 100          |
| 20 ppm          | 23.4                | 126          |
| 10 ppm          | 9.4                 | 102          |
| 5 ppm           | 4.6                 | 100          |
| 2.5 ppm         | 2.5                 | 109          |
| <b>Mean [%]</b> |                     | <b>109</b>   |

## Sausage

| Target Value    | Concentration [ppm] | Recovery [%] |
|-----------------|---------------------|--------------|
| 40 ppm          | 35.1                | 100          |
| 20 ppm          | 21.7                | 123          |
| 10 ppm          | 9.8                 | 111          |
| 5 ppm           | 5.0                 | 113          |
| 2.5 ppm         | 2.5                 | 115          |
| <b>Mean [%]</b> |                     | <b>116</b>   |

For different matrices, the mean linearity is ranging from 90% to 116%. The linearity is independent of the specific concentration and may only be affected by the intra-assay and inter-assay variation as stated in chapter 2.

## 6. Cross-Reactivity

The following cross-reactivities could be determined:

**Table 6: Cross-reactive food matrices in the AlerTox® ELISA Pistachio**

| Raw material | Cross-reactivity |
|--------------|------------------|
| Cashew       | <0.0025          |
| Cayenne      | <0.0004          |
| Chia         | <0.0002          |
| Chili        | <0.0005          |
| Hazelnut     | <0.0002          |
| Pumpkin seed | <0.0002          |
| Poppy seed   | <0.0002          |
| Pepper       | <0.0025          |

For the following foods, no cross-reactivity (results < LOQ) could be detected:

**Table 7: Non-cross-reactive food matrices in the AlerTox® ELISA Pistachio**

|                |              |              |           |                |                 |
|----------------|--------------|--------------|-----------|----------------|-----------------|
| Adzuki         | Celery       | Cress        | Kiwi      | Peach          | Shrimp, raw     |
| Almond         | Cherry       | Cumin        | Lamb      | Peanut         | Shrimps         |
| Apricot        | Chervil      | Duck         | Lentil    | Pecan          | Soy             |
| Barley         | Chestnut     | Egg          | Linseed   | Pine seed      | Soy lecithin    |
| Bean, white    | Chickpea     | Ewe's milk   | Lupin     | Plum           | Split peas      |
| Beef           | Cocoa        | Fenugreek    | Macadamia | Pork           | Sunflower seeds |
| Bovine gelatin | Coconut      | Fish gelatin | Mustard   | Potato         | Tomato          |
| Brazil nut     | Cod          | Gliadin      | Nutmeg    | Rice           | Turkey          |
| Buckwheat      | Corn         | Goat's milk  | Oats      | Rye            | Walnut          |
| Caraway        | Cow's milk   | Guar gum     | Onion     | Saccharose     | Wheat           |
| Carob gum      | Crab, cooked | Isinglass    | Paprika   | Sesame         |                 |
| Carrot         | Crab, raw    | Kidney bean  | Pea       | Shrimp, cooked |                 |

## 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 cookie sample and a sample spiked with 10 ppm of pistachio 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 8: Variation of extraction temperature in the AlerTox® ELISA Pistachio**

| Sample        | Results 60 °C | Results 25 °C | Results 40 °C | Results 70 °C |
|---------------|---------------|---------------|---------------|---------------|
| Cookie 0 ppm  | 0.06 ppm      | 0.03 ppm      | 0.08 ppm      | 0.05 ppm      |
| Cookie 10 ppm | 10.5 ppm      | 12.1 ppm      | 8.0 ppm       | 11.9 ppm      |

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 9: Variation of extraction time in the AlerTox® ELISA Pistachio**

| Sample        | Result 15 min | Result 10 min | Results 20 min |
|---------------|---------------|---------------|----------------|
| Cookie 0 ppm  | 0.06 ppm      | 0.05 ppm      | 0.03 ppm       |
| Cookie 10 ppm | 10.5 ppm      | 11.3 ppm      | 8.9 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 10: Drift in the AlerTox® ELISA Pistachio**

| Sample        | Result 20 min | Result 16 min | Results 24 min |
|---------------|---------------|---------------|----------------|
| Cookie 0 ppm  | 0.06 ppm      | 0.00 ppm      | 0.00 ppm       |
| Cookie 10 ppm | 10.5 ppm      | 6.8 ppm       | 13.2 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.