# Validation Report
# *SENSISpec Pistachio ELISA (Cat. No. HU0030021)*

Version 4

## CONTENT

## 1. Scope

The **SENSI*Spec* Pistachio ELISA** is designed for the sensitive determination of pistachio in food, based on a polyclonal antibody. The present report describes the validation process and summarizes its results.

## 2. Precision

### 2.1. Repeatability

Repeatability (intra-assay variation) was determined by testing three controls of various concentration levels in 24fold replicates, either in sample diluent and cookie matrix. After identification of possible outliers the following results were obtained.

**Table 1: Repeatability of the SENSIS*pec* Pistachio ELISA in sample diluent**

| Replicate | 1 ppm | 4 ppm | 20 ppm | |
|---|---|---|---|---|
| 1 | 0.98 | 3.90 | 18.7 | |
| 2 | 0.96 | 4.08 | 22.7 | |
| 3 | 0.97 | 3.98 | 22.6 | |
| 4 | 1.03 | 4.38 | 22.5 | |
| 5 | 1.02 | 4.10 | 19.4 | |
| 6 | 1.01 | 4.04 | 19.1 | |
| 7 | 0.97 | 3.86 | 19.1 | |
| 8 | 0.86 | 4.14 | 19.4 | |
| 9 | 0.87 | 3.95 | 21.8 | |
| 10 | 0.84 | 4.33 | 22.5 | |
| 11 | 0.86 | 4.06 | 18.8 | |
| 12 | 0.90 | 4.39 | 19.6 | |
| 13 | 0.87 | 4.06 | 19.2 | |
| 14 | 0.94 | 4.20 | 20.3 | |
| 15 | 0.96 | 4.00 | 23.9 | |
| 16 | 0.96 | 4.27 | 22.2 | |
| 17 | 1.00 | 3.99 | 19.2 | |
| 18 | 0.97 | 4.18 | 18.8 | |
| 19 | 0.89 | 4.21 | 20.4 | |
| 20 | 0.96 | 4.08 | 23.1 | |
| 21 | 0.94 | 3.89 | 21.5 | |
| 22 | 0.92 | 4.04 | 18.7 | |
| 23 | 0.92 | 3.91 | 24.8 | |
| 24 | 0.93 | 4.11 | 17.0 | |
| **Mean** | 0.94 | 4.09 | 20.6 | |
| **SD** | 0.05 | 0.15 | 2.02 | **Mean** |
| **CV [%]** | **5.7** | **3.7** | **9.8** | **6.4** |

The coefficient of variation in sample diluent is ranging from 3.7% to 9.8% depending on the concentration.

**Table 2: Repeatability of the SENSI*spec* Pistachio ELISA in cookie matrix**

| Replicate | 1 ppm | 4 ppm | 20 ppm | |
|---|---|---|---|---|
| 1 | 1.01 | 4.15 | 20.6 | |
| 2 | 1.01 | 4.39 | 21.6 | |
| 3 | 1.06 | 4.66 | 23.6 | |
| 4 | 1.16 | 4.43 | 21.2 | |
| 5 | 1.13 | 4.64 | 19.7 | |
| 6 | 1.07 | 4.53 | 20.3 | |
| 7 | 1.00 | 4.38 | 19.2 | |
| 8 | 1.02 | 4.27 | 20.3 | |
| 9 | 1.00 | 4.48 | 19.7 | |
| 10 | 1.01 | 4.71 | 20.3 | |
| 11 | 1.05 | 4.77 | 24.0 | |
| 12 | 1.04 | 4.48 | 24.3 | |
| 13 | 1.01 | 4.31 | 22.7 | |
| 14 | 0.96 | 4.42 | 18.0 | |
| 15 | 1.05 | 4.10 | 20.6 | |
| 16 | 1.01 | 4.16 | 19.5 | |
| 17 | 1.02 | 4.27 | 20.6 | |
| 18 | 0.99 | 4.40 | 23.6 | |
| 19 | 1.04 | 4.57 | 24.9 | |
| 20 | 1.01 | 4.27 | 22.3 | |
| 21 | 1.03 | 4.20 | 19.0 | |
| 22 | 0.97 | 4.28 | 20.4 | |
| 23 | 1.01 | 4.46 | 17.6 | |
| 24 | 0.97 | 4.44 | 19.4 | |
| **Mean** | 1.03 | 4.41 | 21.0 | |
| **SD** | 0.05 | 0.18 | 2.00 | **Mean** |
| **CV [%]** | **4.5** | **4.1** | **9.5** | **6.0** |

The coefficient of variation in cookie matrix is ranging from 4.1% to 9.5% depending on the concentration.

## 2.2. Reproducibility

Reproducibility (inter-assay variation) was determined by testing three controls of various concentration levels in four different test runs of the same kit lot, either in sample diluent and cookie matrix.

**Table 3: Reproducibility of the SENSI*Spec* Pistachio ELISA in sample diluent**

| Assay No. | 1 ppm | 4 ppm | 20 ppm | |
|---|---|---|---|---|
| **1** | 0.84 | 3.79 | 17.9 | |
| **2** | 0.97 | 4.20 | 20.8 | |
| **3** | 0.86 | 4.16 | 22.2 | |
| **4** | 1.03 | 4.05 | 19.5 | |
| **Mean** | 0.93 | 4.05 | 20.1 | |
| **SD** | 0.09 | 0.18 | 1.84 | **Mean** |
| **CV [%]** | **9.7** | **4.5** | **9.2** | **7.8** |

The coefficient of variation in sample diluent is ranging from 4.5% to 9.7% depending on the concentration.

**Table 4: Reproducibility of the SENSI*Spec* Pistachio ELISA in cookie matrix**

| Assay No. | Level 1 | Level 2 | Level 3 | |
|---|---|---|---|---|
| **1** | 0.79 | 3.97 | 23.3 | |
| **2** | 0.82 | 3.72 | 22.0 | |
| **3** | 0.80 | 3.27 | 19.3 | |
| **4** | 1.01[1)] | 3.63 | 19.2 | |
| **Mean** | 0.80 | 3.65 | 20.9 | |
| **SD** | 0.01 | 0.29 | 2.03 | **Mean** |
| **CV [%]** | **1.7** | **7.9** | **9.7** | **6.4** |

[1)] identified as outlier by 1.5 IQR testing; not considered in the calculation

The coefficient of variation in cookie matrix is ranging from 1.7% to 9.7% depending on the concentration.

## 2.3. Reproducibility of Extraction

For determining the reproducibility of the extraction process pistachio proficiency test material (DLA 07/2019, sample A) was applied. The homogeneity of the material was assured by the proficiency test provider by analyzing 20 different samples of the material with 3 different test kits, respectively. According PT report the coefficient of variation was ranging from 3.8% to 5.5%.

The material was extracted ten times. The coefficient of variation was calculated by applying the mean results of every extract.

**Table 5: Reproducibility of extraction of the SENSI*Spec* Pistachio ELISA**

| Extraction No. | Mean Result [ppm] |
|---|---|
| **1** | 36.4 |
| **2** | 38.4 |
| **3** | 39.0 |
| **4** | 38.9 |

| | |
|---|---|
| **5** | 34.4 |
| **6** | 39.6 |
| **7** | 42.6 |
| **8** | 38.1 |
| **9** | 37.0 |
| **10** | 39.9 |
| **Mean** | 38.4 |
| **SD** | 2.21 |
| **CV%** | **5.8** |

The coefficient of variation is 5.8% for the inter-extraction reproducibility trial.

## 3. Analytical Sensitivity

For determination of the analytical sensitivity (LOD), sample diluent and various pistachio free representative sample matrices 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 6a: Matrix-dependent and matrix-independent analytical sensitivity of the Immunolab Pistachio ELISA (complete data)**

| Replicate | Sample diluent [OD] | Cookie matrix [OD] | Sweets matrix [OD] | Ice cream matrix [OD] | Chocolate[1)] matrix [OD] | Spice[1)] matrix [OD] | Food supplement matrix [OD] |
|---|---|---|---|---|---|---|---|
| 1 | 0.059 | 0.114 | 0.063 | 0.072 | 0.116 | 0.199 | 0.118 |
| 2 | 0.058 | 0.125 | 0.064 | 0.081 | 0.118 | 0.220 | 0.118 |
| 3 | 0.058 | 0.125 | 0.065 | 0.074 | 0.120 | 0.240 | 0.114 |
| 4 | 0.059 | 0.133 | 0.065 | 0.088 | 0.130 | 0.220 | 0.122 |
| 5 | 0.059 | 0.134 | 0.067 | 0.077 | 0.126 | 0.254 | 0.126 |
| 6 | 0.056 | 0.131 | 0.064 | 0.076 | 0.120 | 0.233 | 0.121 |
| 7 | 0.051 | 0.122 | 0.074[2)] | 0.068 | 0.112 | 0.221 | 0.121 |
| 8 | 0.053 | 0.115 | 0.066 | 0.067 | 0.102 | 0.205 | 0.129 |
| 9 | 0.060 | 0.130 | 0.066 | 0.069 | 0.112 | 0.250 | 0.115 |
| 10 | 0.062 | 0.133 | 0.060 | 0.086 | 0.122 | 0.205 | 0.119 |
| 11 | 0.059 | 0.124 | 0.068 | 0.076 | 0.118 | 0.222 | 0.121 |
| 12 | 0.059 | 0.128 | 0.070 | 0.083 | 0.117 | 0.226 | 0.122 |
| 13 | 0.061 | 0.132 | 0.066 | 0.079 | 0.124 | 0.247 | 0.121 |
| 14 | 0.054 | 0.133 | 0.065 | 0.080 | 0.119 | 0.247 | 0.126 |
| 15 | 0.049 | 0.109 | 0.062 | 0.067 | 0.111 | 0.230 | 0.128 |
| 16 | 0.062 | 0.135 | 0.067 | 0.075 | 0.109 | 0.224 | 0.129 |
| 17 | 0.061 | 0.134 | 0.069 | 0.070 | 0.112 | 0.203 | 0.111 |
| 18 | 0.057 | 0.134 | 0.063 | 0.076 | 0.119 | 0.204 | 0.117 |
| 19 | 0.061 | 0.128 | 0.064 | 0.079 | 0.117 | 0.201 | 0.116 |
| 20 | 0.059 | 0.134 | 0.068 | 0.075 | 0.121 | 0.219 | 0.116 |
| 21 | 0.048 | 0.126 | 0.063 | 0.071 | 0.118 | 0.228 | 0.125 |
| 22 | 0.050 | 0.122 | 0.065 | 0.074 | 0.118 | 0.233 | 0.121 |
| 23 | 0.050 | 0.113 | 0.062 | 0.076 | 0.112 | 0.214 | 0.123 |

| Replicate | Sample diluent [OD] | Cookie matrix [OD] | Sweets matrix [OD] | Ice cream matrix [OD] | Chocolate[1)] matrix [OD] | Spice[1)] matrix [OD] | Food supplement matrix [OD] |
|---|---|---|---|---|---|---|---|
| 24 | 0.055 | 0.121 | 0.063 | 0.078 | 0.111 | 0.204 | 0.117 |
| **Mean** | **0.057** | **0.126** | **0.065** | **0.076** | **0.117** | **0.223** | **0.121** |
| **SD** | **0.004** | **0.008** | **0.002** | **0.006** | **0.006** | **0.017** | **0.005** |
| **LOD [ppm]** | **0.07** | **0.42** | **0.03** | **0.16** | **0.28** | **0.84** | **0.27** |
| Corresponding Calibration Curve data [OD] | | | | | | | |
| **0 ppm** | 0.057 | 0.058 | 0.065 | 0.060 | 0.065 | 0.067 | 0.070 |
| **1 ppm** | 0.277 | 0.277 | 0.317 | 0.270 | 0.317 | 0.312 | 0.313 |
| **4 ppm** | 0.721 | 0.721 | 0.825 | 0.711 | 0.825 | 0.791 | 0.788 |
| **10 ppm** | 1.198 | 1.198 | 1.313 | 1.193 | 1.313 | 1.307 | 1.272 |
| **40 ppm** | 1.836 | 1.836 | 2.103 | 1.794 | 2.103 | 2.100 | 1.929 |

[1)] addition of 1% PVP to sample extraction buffer prior extraction
[2)] identified as outlier by 1.5 IQR testing; not considered in the calculation

Note: In some cases. various samples of the same kind (e.g. different cookies) were tested. The table above represents the matrix with the highest LOD. Anyway, the table below summarizes all results.

**Table 6b: Matrix-dependent and matrix-independent analytical sensitivity of the Immunolab Pistachio ELISA (summary)**

| Matrix Type | Variant | LOD |
|---|---|---|
| Sample diluent | N/A | 0.07 ppm |
| Cookies | “Spoon Biscuit” | 0.06 ppm |
| | “TUC” cookie | 0.06 ppm |
| | “Lemon cookie” | 0.42 ppm |
| Ice-cream | Type Vanilla | 0.02 ppm |
| | Type Lab sample | 0.00 ppm |
| | Type Strawberry | 0.16 ppm |
| Sweets | Vitamin drop | 0.04 ppm |
| | Chewy candy | 0.00 ppm |
| Chocolate[1)] | Chocolate bar | 0.02 ppm |
| | Type 1, 59% cocoa | 0.15 ppm |
| | Type 2, 72% cocoa | 0.28 ppm |
| Spices[1)] | Curcuma | 0.07 ppm |
| | Paprika | 0.84 ppm |
| | Chili | 0.73 ppm |
| | Cumin | 0.20 ppm |
| Food supplement | “Almased” | 0.07 ppm |
| | “Wellmix Classic” | 0.21 ppm |
| | “Wellmix Vanilla” | 0.11 ppm |

[1)] addition of 1% PVP to sample extraction buffer prior extraction

With respect to the sample matrix limits of detection vary from 0.00 to 0.84 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 lowest positive standard (1 ppm) was defined as limit of quantification to assure that all uncontaminated matrices result in concentrations lower than this value.

## 4. Recovery

### 4.1. Food Samples

For recovery experiments different sample matrices were spiked with pistachio extract to obtain various final concentrations after performing all sample pre-treatment steps. The result of the un-spiked matrix was subtracted before evaluation. Tested samples and calculated results were as follows.

**Table 7: Recovery of various samples tested with the SENSI*Spec* Pistachio ELISA**

***Cookie, type "Spoon biscuit", Zero level: 0.00 ppm***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.88 | 88 |
| **4 ppm** | 3.41 | 85 |
| **10 ppm** | 8.17 | 82 |
| | **Mean** | **85** |

***Cookie, type "TUC", Zero level: 0.01 ppm***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.95 | 95 |
| **4 ppm** | 3.84 | 96 |
| **10 ppm** | 9.43 | 94 |
| | **Mean** | **95** |

***Cookie, type "Lemon Cookie", Zero level: 0.18 ppm***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 1.00 | 100 |
| **4 ppm** | 3.66 | 92 |
| **10 ppm** | 7.90 | 79 |
| | **Mean** | **90** |

***Sweets, type "Vitamin drop", Zero level: 0.00 ppm[1)]***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.85 | 85 |
| **4 ppm** | 3.40 | 85 |
| **10 ppm** | 7.61 | 76 |
| | **Mean** | **82** |

***Sweets, type "Chewy candy", Zero level: 0.00 ppm[1)]***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.76 | 76 |
| **4 ppm** | 3.09 | 77 |
| **10 ppm** | 7.01 | 70 |
| | **Mean** | **74** |

***Ice-cream, type Lab sample, Zero level: 0.00 ppm***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.69 | 69 |
| **4 ppm** | 3.07 | 77 |
| **10 ppm** | 9.07 | 91 |
| | **Mean** | **79** |

***Ice-cream, type "Vanilla", Zero level: 0.00 ppm***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 1.05 | 105 |
| **4 ppm** | 3.42 | 86 |
| **10 ppm** | 9.65 | 97 |
| | **Mean** | **96** |

***Ice-cream, type "Strawberry", Zero level: 0.00 ppm***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.85 | 85 |
| **4 ppm** | 3.27 | 82 |
| **10 ppm** | 8.00 | 80 |
| | **Mean** | **82** |

***Chocolate, type Chocolate bar, Zero level: 0.03 ppm[1)]***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.81 | 81 |
| **4 ppm** | 3.32 | 83 |
| **10 ppm** | 7.55 | 76 |
| | **Mean** | **80** |

***Chocolate, type 59% Cocoa, Zero level: 0.12 ppm[1)]***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.83 | 83 |
| **4 ppm** | 3.52 | 88 |
| **10 ppm** | 8.26 | 83 |
| | **Mean** | **84** |

***Chocolate, type 72% Cocoa, Zero level: 0.11 ppm[1)]***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.79 | 79 |
| **4 ppm** | 3.31 | 83 |
| **10 ppm** | 7.01 | 70 |
| | **Mean** | **77** |

***Spices, Curcuma, Zero level: 0.00 ppm[1)]***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.73 | 73 |
| **4 ppm** | 3.06 | 77 |
| **20 ppm** | 6.91 | 69 |
| | **Mean** | **73** |

***Spices, Cumin, Zero level: 0.18 ppm[1)]***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.70 | 70 |
| **4 ppm** | 2.81 | 70 |
| **10 ppm** | 10.69 | 107 |
| | **Mean** | **82** |

***Spices, Chili, Zero level: 0.57 ppm[1)]***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.90 | 90 |
| **4 ppm** | 3.42 | 86 |
| **10 ppm** | 7.95 | 80 |
| | **Mean** | **85** |

***Spices, Paprika, Zero level: 0.00 ppm[1)]***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.86 | 86 |
| **4 ppm** | 3.44 | 86 |
| **10 ppm** | 6.77 | 68 |
| | **Mean** | **80** |

***Food supplement, type "Almased", Zero level: 0.17 ppm***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.67 | 67 |
| **4 ppm** | 3.06 | 76 |
| **10 ppm** | 7.14 | 71 |
| | **Mean** | **72** |

***Food supplement, type "Wellmix Classic", Zero level: 0.10 ppm***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.66 | 66 |
| **4 ppm** | 2.52 | 63 |
| **10 ppm** | 8.28 | 83 |
| | **Mean** | **70** |

***Food supplement, type "Wellmix Vanilla", Zero level: 0.05 ppm***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **1 ppm** | 0.71 | 71 |
| **4 ppm** | 2.72 | 68 |
| **10 ppm** | 8.32 | 83 |
| | **Mean** | **74** |

[1)] addition of 1% PVP to sample extraction buffer prior extraction

Mean recoveries are ranging from **70**% to **96**% depending on the sample matrix.

### 4.1.1. Heat-treated Matrices

To exemplarily assess changes in analytical sensitivity and recovery for processed food samples, a cookie matrix was spiked with 10 ppm of pistachio and divided in two parts. One part was baked at 150 °C for 20 min. Processed as well as unprocessed extracts were tested undiluted as well as in various dilutions. Additionally, non-spiked zero matrix was tested. All tests were performed in duplicates.

**Table 8: Accuracy (heat-treated matrices) of the SENSI*Spec* Pistachio ELISA**

| Matrix | Level 1 [0 ppm] | Level 4 [1.25 ppm] | Level 5 [2.5ppm] | Level 6 [5 ppm] | Level 7 [10 ppm] |
|---|---|---|---|---|---|
| Cookie / unprocessed | 0.075 | 1.42 | 2.79 | 5.92 | 10.7 |
| Cookie / processed | 0.052 | 0.66 | 1.39 | 2.70 | 5.23 |

Due to the baking process recovery decreases by ~50%.

## 4.2. Rinse water /CIP

The sensitivity for rinse water / CIP was determined by diluting pistachio extract in different commonly applied water-based rinse solutions to various concentrations. The pH was adjusted to 8.2 ± 0.5 before running the test. Afterwards the samples were diluted 1:5 in pre-diluted sample extraction buffer. Tested samples and calculated results were as follows.

**Table 9: Recovery of the SENSI*Spec* Pistachio ELISA for Rinse Solutions**

***Pure water, Zero level 0.00 mg/L***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **0.25 mg/L** | 0.22 | 88 |
| **0.5 mg/L** | 0.46 | 93 |
| **1 mg/L** | 0.93 | 93 |
| **2 mg/L** | 1.99 | 99 |
| **4 mg/L** | 3.45 | 86 |
| | **Mean** | **92** |

***0.5% Sodium triphosphate, Zero level 0.00 mg/L***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **0.25 mg/L** | 0.23 | 91 |
| **0.5 mg/L** | 0.35 | 70 |
| **1 mg/L** | 0.76 | 76 |
| **2 mg/L** | 1.78 | 89 |
| **4 mg/L** | 4.26 | 107 |
| | **Mean** | **87** |

***2% NaOH, Zero level 0.00 mg/L***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **0.25 mg/L** | 0.20 | 79 |
| **0.5 mg/L** | 0.48 | 97 |
| **1 mg/L** | 0.92 | 92 |
| **2 mg/L** | 1.52 | 76 |
| **4 mg/L** | 2.59 | 65 |
| | **Mean** | **82** |

***0.5% Nitric acid, Zero level 0.00 mg/L***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **0.25 mg/L** | 0.23 | 92 |
| **0.5 mg/L** | 0.43 | 86 |
| **1 mg/L** | 0.90 | 90 |
| **2 mg/L** | 1.89 | 94 |
| **4 mg/L** | 3.84 | 96 |
| | **Mean** | **92** |

***0.5% Citric acid, Zero level 0.0 mg/L***

| Target Value | Actual Concentration | Recovery [%] |
|---|---|---|
| **0.25 mg/L** | 0.26 | 106 |
| **0.5 mg/L** | 0.48 | 97 |
| **1 mg/L** | 0.86 | 86 |
| **2 mg/L** | 2.06 | 103 |
| **4 mg/L** | 4.69 | 117 |
| | **Mean** | **102** |

Mean recoveries for rinse water are ranging from **82**% to **102**% depending on the kind of rinse solution.

### 4.3. Swabs

To monitor the swab performance, surfaces (5 cm * 5 cm) were contaminated with defined amounts of a pistachio (in solution). Each amount was applied in duplicate. The surfaces were allowed to dry completely. The swabs were moistened in 1 mL pre-diluted sample extraction buffer. Then the surfaces were swabbed thoroughly. After swabbing the swabs were redissolved in the initial 1 mL of buffer. After redissolving 100 µL of the solutions were applied in the ELISA. The mean results were recalculated to the amount of pistachio / area.

**Table 10: Recovery of the SENSI*Spec* Pistachio ELISA for Swabs**

***Swabs, Zero level 0.00 ng / cm²***

| Target Value [ng / cm²] | Test Result [ng / cm²] | CV [%] | Recovery [%] |
|---|---|---|---|
| **20** | 15.9 | 4.9 | 80 |
| **60** | 42.8 | 10 | 71 |
| **200** | 147 | 39 | 73 |
| | **Mean** | **18** | **75** |

Recovery is ranging from 71-80%. Mean recovery is 75%. Repeated swabbing of the same surface indicates that varying recoveries are an intrinsic feature of the method which can only be improved by multiple repetitions. Recoveries are affected by solubility of proteins, residual liquid and complete swabbing of the surface area.

Note that surfaces were contaminated by protein extracts and not complete commodities. As the latter are more particulate by nature they might be swabbed more easily. Thus, the approach chosen here reflects a worst-case scenario.

## 5. Linearity

Linearity was determined by spiking various matrices with pistachio extract 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 percent of this reference after consideration of the dilution factor. Regarding the matrices extracted by addition of PVP, further dilution was applied with standard extraction buffer without PVP.

**Table 11: Matrix dependent linearity of the SENSI*Spec* Pistachio ELISA**

***Spoon Cookie***

| Target Value | Concentration [ppm] | Recovery [%] |
|---|---|---|
| **40 ppm** | 38.1 | 100 |
| **20 ppm** | 19.2 | 101 |
| **10 ppm** | 9.57 | 100 |
| **5 ppm** | 5.07 | 107 |
| **2.5 ppm** | 2.66 | 112 |
| | **Mean** | **105** |

***Vitamin drop***

| Target Value | Concentration [ppm] | Recovery [%] |
|---|---|---|
| **40 ppm** | 33.6 | 100 |
| **20 ppm** | 19.2 | 114 |
| **10 ppm** | 10.2 | 122 |
| **5 ppm** | 5.02 | 119 |
| **2.5 ppm** | 2.50 | 119 |
| | **Mean** | **119** |

***Ice-cream, Type Vanilla***

| Target Value | Concentration [ppm] | Recovery [%] |
|---|---|---|
| **40 ppm** | 31.8 | 100 |
| **20 ppm** | 18.6 | 117 |
| **10 ppm** | 7.62 | 96 |
| **5 ppm** | 4.08 | 103 |
| **2.5 ppm** | 2.29 | 115 |
| | **Mean** | **108** |

***Chocolate bar[1)]***

| Target Value | Concentration [ppm] | Recovery [%] |
|---|---|---|
| **40 ppm** | 35.2 | 100 |
| **20 ppm** | 21.1 | 120 |
| **10 ppm** | 10.0 | 114 |
| **5 ppm** | 5.33 | 121 |
| **2.5 ppm** | 2.48 | 113 |
| | **Mean** | **117** |

***Spices, Curcuma[1)]***

| Target Value | Concentration [ppm] | Recovery [%] |
|---|---|---|
| **40 ppm** | 36.0 | 100 |
| **20 ppm** | 20.0 | 111 |
| **10 ppm** | 9.21 | 102 |
| **5 ppm** | 5.54 | 123 |
| **2.5 ppm** | 2.81 | 125 |
| | **Mean** | **116** |

***Food Supplement "Almased"***

| Target Value | Concentration [ppm] | Recovery [%] |
|---|---|---|
| **40 ppm** | 31.3 | 100 |
| **20 ppm** | 15.3 | 98 |
| **10 ppm** | 7.53 | 96 |
| **5 ppm** | 3.65 | 93 |
| **2.5 ppm** | 1.99 | 102 |
| | **Mean** | **97** |

[1)] addition of 1% PVP to sample extraction buffer prior extraction

For different matrices the mean linearity is ranging from 97% to 119%. The linearity is independent of the specific concentration and may only be affected by the precisions stated in chapter 2.

## 6. Cross-Reactivity

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

**Table 12: Non-cross-reactive food matrices in the SENSI*Spec* Pistachio ELISA**

| Raw material | c [ppm] | Raw material | c [ppm] |
|---|---|---|---|
| Adzuki bean | 0.16 | Goat's milk | 0.34 |
| Almond | 0.43 | Guar gum | 0.13 |
| Apricot | 0.53 | Gum arabic | 0.86 |
| Barley | 0.17 | Hazelnut | 0.99 |
| Bean, white | 0.36 | Kidney bean | 0.12 |
| Beef | 0.09 | Kiwi | 0.03 |
| Bovine gelatin | 0.06 | Lamb | 0.03 |
| Brazil nut | 0.17 | Leek | 0.41 |
| Buckwheat | 0.48 | Lentil | 0.67 |
| Cabbage, white | 0.68 | Lupin | 0.17 |
| Caraway | 0.06 | Macadamia | 0.95 |
| Cardamom | 0.14 | Nutmeg | 0.10 |
| Carob gum | 0.16 | Oats | 0.10 |
| Carrot | 0.08 | Onion | 0.40 |
| Cayenne | 0.19 | Paprika | 0.18 |
| Celery | 0.08 | Pea | 0.45 |
| Cherry | 0.17 | Peach | 0.03 |
| Chestnut | 0.21 | Peanut | 0.17 |
| Chia | 0.63 | Pecan | 0.53 |
| Chicken | 0.53 | Pepper, black | 0.43 |
| Chickpea | 0.16 | Pine seed | 0.03 |
| Chili | 0.65 | Pork | 0.14 |
| Cinnamon | 0.07 | Potato | 0.15 |
| Clove | 0.39 | Prawn | 0.09 |
| Cocoa | 0.03 | Pumpkin seed | 0.35 |
| Coconut | 0.12 | Radish | 0.57 |

| Raw material | c [ppm] |
|---|---|
| Cod | 0.20 |
| Corn | 0.15 |
| Cow' milk | 0.14 |
| Cumin | 0.20 |
| Dill | 0.22 |
| Duck | 0.45 |
| Egg, dried | 0.14 |
| Fennel | 0.84 |
| Fenugreek | 0.70 |
| Garden cress | 0.48 |
| Garlic, fresh | 0.69 |
| Garlic, granulated | 0.52 |
| Ginger, fresh | 0.10 |
| Ginger, ground | 0.07 |
| Gliadin | 0.09 |

| Raw material | c [ppm] |
|---|---|
| Rice | 0.49 |
| Rye | 0.28 |
| Saccharose | 0.41 |
| Shrimps | 0.21 |
| Soy flour | 0.30 |
| Soy lecithin | 0.07 |
| Split pea | 0.97 |
| Sunflower seed | 0.30 |
| Thyme | 0.14 |
| Tofu | 0.23 |
| Tomato | 0.13 |
| Turkey | 0.00 |
| Turmeric | 0.02 |
| Walnut | 0.82 |
| Wheat | 0.21 |

The following cross-reactivities could be determined:

**Table 13: Cross-reactive food matrices in the SENSI*Spec* Pistachio ELISA**

| Raw material | c [ppm] | Cross-reactivity [%] |
|---|---|---|
| Poppy seed | 1.06 | 0.0001 |
| Rapeseed | 1.71 | 0.0002 |
| Sesame | 1.02 | 0.0001 |
| Horseradish | 1.17 | 0.0001 |
| Flaxseed | 1.03 | 0.0001 |
| Cashew | 33.3 | 0.0033 |
| Mustard, yellow | 1.64 | 0.0002 |

## 7. Robustness

Robustness was determined by variation of different handling parameters as defined in the instruction manual. The results were compared to the results of samples analyzed according to the intended method. An un-spiked cookie sample and a sample spiked with pistachio extract were analyzed, respectively.

### 7.1. Variation of extraction temperature

The extraction temperature defined as 60°C was changed to 25°C, 40°C and 70°C. respectively using spiked and pure cookie matrix.

**Table 14: Variation of extraction temperature in the SENSI*Spec* Pistachio ELISA**

| Sample | Result 25°C | Result 40°C | Result 60°C | Result 70°C |
|---|---|---|---|---|
| Cookie 0 ppm | < 1 ppm | < 1 ppm | < 1 ppm | < 1 ppm |
| Cookie 10 ppm | 11.9 ppm | 9.06 ppm | 10.5 ppm | 8.33 ppm |

Under consideration of the precision data as stated in chapter 2 the results do not differ significantly.

### 7.2. Variation of extraction time

The extraction time defined as 15 min was changed to 10 min and 20 min respectively.

**Table 15: Variation of extraction time in the SENSI*Spec* Pistachio ELISA**

| Sample | Result 10 min | Result 15 min | Result 20 min |
|---|---|---|---|
| Cookie 0 ppm | < 1 ppm | < 1 ppm | < 1 ppm |
| Cookie 10 ppm | 9.40 | 8.47 | 9.06 |

Under consideration of the precision data as stated in chapter 2 the results do not differ significantly.

### 7.3. 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.

**Table 16: Drift in the SENSI*Spec* Pistachio ELISA**

| Sample | Result 16 min | Result 20 min | Result 24 min |
|---|---|---|---|
| Cookie 0 ppm | < 1 ppm | < 1 ppm | < 1 ppm |
| Cookie 10 ppm | 7.53 ppm | 10.1 ppm | 11.8 ppm |

The results do not differ significantly. Nevertheless, 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.