

Validation REPORT

AgraQuant® Pistachio (COKAL2748)

Sandwich enzyme-linked immunosorbent assay (ELISA) for quantitative determination of Pistachio in food.

Limit of Detection: 0.13ppm

Standard Range: 1-40ppm

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1. Scope

The **AgraQuant® Pistachio ELISA** is designed for the determination of pistachio in food. The present report describes the validation process and its results.

2. Precision

2.1. Intra-Assay Variation

The intra-assay variation was determined by testing three controls of various concentration levels in 20fold replicates.

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	
Mean	4.0	8.4	36.6	
SD	0.3	0.7	2.2	Mean
CV [%]	8.1	8.0	6.0	7.3

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

2.2. 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.

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	
Mean	4.3	9.6	37.3	
SD	0.2	1.1	3.8	Mean
VK [%]	5.2	11.9	10.3	9.2

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.

Cookie

Target Value	Actual Concentration	Recovery [%]
4 ppm	3.4	85
10 ppm	10.2	102
40 ppm	37.8	94
	Mean	94

Cornflakes

Target Value	Actual Concentration	Recovery [%]
4 ppm	3.6	89
10 ppm	10.0	100
40 ppm	38.9	97
	Mean	96

Ice-cream

Target Value	Actual Concentration	Recovery [%]
4 ppm	4.0	100
10 ppm	8.2	82
40 ppm	34.1	85
	Mean	89

Chocolate

Target Value	Actual Concentration	Recovery [%]
4 ppm	3.1	78
10 ppm	8.4	84
40 ppm	32.5	81
	Mean	81

Sausage

Target Value	Actual Concentration	Recovery [%]
4 ppm	3.6	89
10 ppm	9.3	93
40 ppm	35.1	88
	Mean	90

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:

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
Mean	0.043	0.039	0.035	0.045	0.033	0.022
SD	0.006	0.009	0.004	0.008	0.006	0.003
Limit of Detection	0.13 ppm	0.18 ppm	0.01 ppm	0.20 ppm	0.09 ppm	0.17 ppm

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 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 percent of this reference after consideration of the dilution factor.

Cookie

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
	Mean [%]	99

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
	Mean [%]	90

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
	Mean [%]	109

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
	Mean [%]	109

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
	Mean [%]	116

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:

Raw material	Cross-reactivity
Walnut	0.0008%
Sunflower seed	0.0002%
Cashew	12%
Pecan	0.0005%
Hazelnut	0.17%

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

Almond	Cherry	Guar gum	Plum	Shrimps, uncooked
Apricot	Chestnut	Kiwi	Poppy seed	Soy
Barley	Chicken	Lentil	Pork meat	Soy lecithin
Bean	Chickpea	Lupin	Potato	Tomato
Beef	Cocoa	Macadamia	Pumpkin seed	Wheat
Bovine gelatin	Coconut	Mustard	Rice	
Brazil nut	Cod	Oats	Rye	
Carob gum	Corn	Pea	Saccharose	
Carrot	Cow's milk	Peanut	Sesame	
Celery	Egg	Pine seed	Shrimps, 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.

Variation of extraction temperature

The extraction temperature, defined as 60°C, was changed to 25°C, 40°C and 70°C, respectively.

Sample	Result 60°C	Result 25°C	Result 40°C	Result 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, inter-assay and inter-extraction variations the results do not differ significantly.

Variation of extraction time

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

Sample	Result 15 min	Result 10 min	Result 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, inter-assay and inter-extraction variations the results do not differ significantly

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).

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